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Dielectric Constants of Crystalline Powders at Microwave Frequencies

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Dielectric constants of alkali halide crystals have been measured in the 10 cm. microwave region by the standing wave technique using the method of mixtures. Ionic polarization has been determined for the crystals and from it Szigeti's short range correction factor *S* has been calculated. A good agreement has been observed between Szigeti's and the authors' values for the correction factor *S*. The dielectric constants of sodium halides are greater than those of potassium halides and the dielectric constants of these halides show a regularly increasing trend from chloride via bromide to iodide. This behaviour is explained in terms of the ionic polarization of these crystals.

THE determination of the dielectric constant of crystalline powders at microwave frequencies has been a problem. Though several methods can be adopted, such as growing a complete crystal of cubical size required for measurement with the standing wave technique, in some cases it becomes difficult to grow such a large crystal especially for carrying out measurements at the 10 cm. region. To overcome such difficulties, the method of mixtures has been used in the present studies.

Several empirical and derived formulae have been suggested[1] for the determination of the dielectric constant of one constituent of a mixture, when the dielectric constant of the binding medium and that of the mixture itself are known. It has been difficult to develop a formula for calculating the dielectric constants of mixtures containing crystalline powders as one of the components. Formulae for calculating the dielectric constants of mixtures of ellipsoidal particles are available[2] but these cannot be applied to the case of the crystalline powders since the dielectric constant also depends on the shape and size of the particles[3]. In the present paper Wiener[4] formula as modified by Fricke[5] has been used.

Theory

Wiener realized that the geometrical shape of dispersed particles in a continuous medium might influence the distribution of the electrical field and consequently the dielectric constant of the mixture. He derived the following formula for calculating the dielectric constant of a mixture:

$$\frac{\epsilon_m-1}{\epsilon_m+u}=v_1\,.\,\frac{\epsilon_1-1}{\epsilon_1+u}+u_2\,\frac{\epsilon_2-1}{\epsilon_2+u}\quad\ldots\ldots\ (1)$$

where ϵ_m, ϵ_1 and ϵ_2 are the dielectric constants of the mixture and of the individual components, u is a constant depending on the shape of the particles, and v_1 and v_2 are the fractional volumes of the components.

Fricke worked out the case where one component is dispersed in the other, the latter being continuous and homogeneous, and derived the following formula:

$$\frac{\epsilon_m-\epsilon_2}{\epsilon_m+u\epsilon_2}=v_1\,.\,\frac{\epsilon_1-\epsilon_2}{\epsilon_1+u\epsilon_2}\quad\ldots\ldots\ldots\ldots\ (2)$$

where u is a factor depending on ϵ_1, ϵ_2 and the shape of particles. Since at microwave frequencies ϵ_1 and ϵ_2 are complex quantities, u is also complex. u being unknown for crystalline particles, it is difficult to solve the equation (2) for ϵ_1.

To avoid such complications, we have used in the present study crystal powders and binding medium of negligible dielectric loss. Paraffin wax has been used as the binding medium having a dielectric loss of 5×10^{-5}. The method of Burton and Turnbull[6] has been used for solving the equation for ϵ_1.

Method for solving equation for ϵ_1

Starting from equation (2) and putting

$$\frac{\epsilon_1 - \epsilon_2}{\epsilon_1 + u\epsilon_2} = C$$

we have

$$\frac{\epsilon_m - \epsilon_2}{\epsilon_m + u\epsilon_2} = Cv_1$$

From this we obtain

$$\log \frac{\epsilon_m - \epsilon_2}{\epsilon_m + u\epsilon_2} = \log v_1 + \log C \quad \ldots\ldots \quad (3)$$

Now if we plot $\log \frac{\epsilon_m - \epsilon_2}{\epsilon_m + u\epsilon_2}$ against $\log v_1$, we get a straight line inclined at an angle of 45 deg., with the abscissa (v_1). This will be true, however, for one value of u only; any other value of u will give lines inclined at angles other than 45 deg.

By assigning different values for u and plotting the graph it is possible to find the proper value of u.

Several values of u (near about 2) were chosen and for each value of u a straight line graph was obtained (Fig. 1). The tangent of each line was evaluated and by plotting u against the tangents obtained a smooth curve was obtained (Fig. 2).

The value of u corresponding to a line, giving a value of the tangent equal to unity, could be read from the graph. This value of u was used to extrapolate the straight line to a volume concentration ($v_1 = 1$). For potassium bromide, the proper value of u was found to be 1·75 and its dielectric constant 4·88.

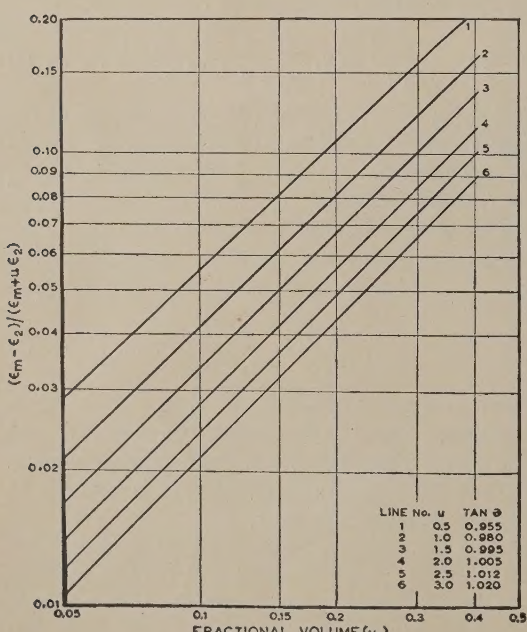

FIG. 1 — STRAIGHT LINE PLOTS OF $(\epsilon_m - \epsilon_2)/(\epsilon_m + u\epsilon_2)$ VERSUS FRACTIONAL VOLUME (v_1) FOR POTASSIUM BROMIDE

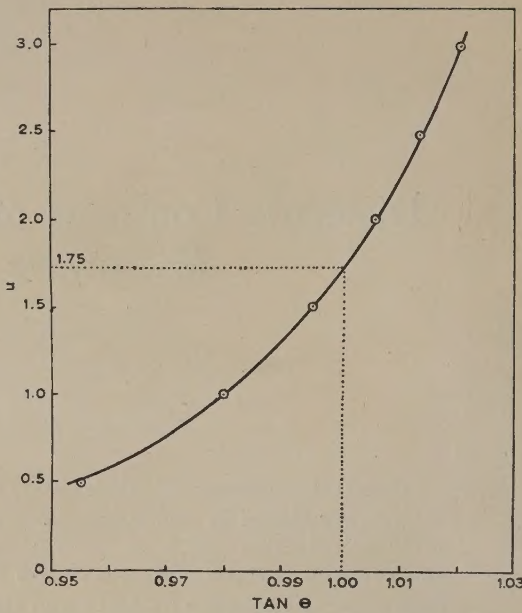

FIG. 2 — INTERPOLATION OF u VALUES FOR POTASSIUM BROMIDE

Since the error arising due to extrapolation is large, we have slightly modified the method of extrapolation to the volume concentration ($v_1 = 1$), and the following method has been adopted.

From the experimental values of ϵ_m up to ($v_1 =$ 0·4), the proper value of u was determined. Using this value of u, a straight line graph between $\frac{\epsilon_m - \epsilon_2}{\epsilon_m + u\epsilon_2}$ and the fractional volume (v_1) was drawn on a log log graph paper and instead of extrapolation to $v_1 = 1$, we interpolated the straight line to $v_1 = 0·1$ (Fig. 3). This value of $\frac{\epsilon_m - \epsilon_2}{\epsilon_m + u\epsilon_2}$ from the graph for $v_1 = 0·1$ gives, on multiplying by 10, the value for $v_1 = 1$. This is simpler because we have only to read the value from the graph and not to extend the straight line up to $v_1 = 1$, which may result in errors.

Now at $v_1 = 1$, ϵ_m becomes ϵ_1 and we have

$$\epsilon_1 = \epsilon_2 \frac{1 + ux}{1 - x} \quad \ldots\ldots\ldots \quad (4)$$

where $x = \frac{\epsilon_m - \epsilon_2}{\epsilon_m + u\epsilon_2}$ (for $v_1 = 1$).

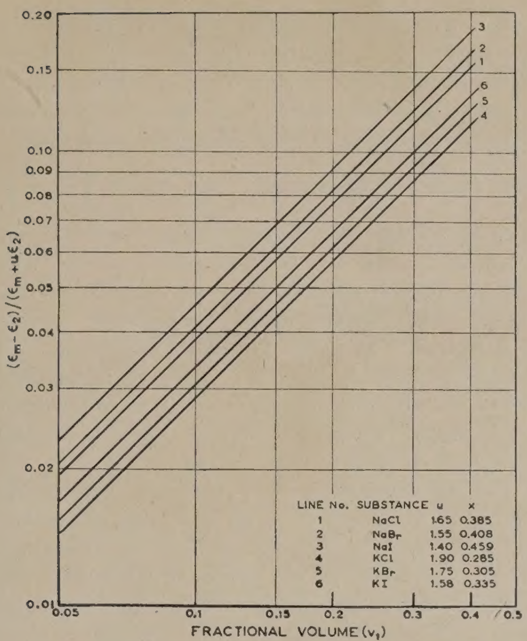

FIG. 3 — INTERPOLATION FOR OBTAINING x VALUES FOR DIFFERENT HALIDES

Experimental procedure

The standing wave technique used in the present study has been described in the previous paper[7]. Since the size and shape of the samples are of the utmost importance for obtaining accurate results, samples were carefully prepared. Paraffin wax was melted and crystal powder was added in known amounts. The vessel was heated at a constant temperature and all air bubbles were removed[8]. The molten mixture was stirred well until the mixture became so viscous that it did not allow the crystalline particles to settle down in liquid paraffin wax. The molten mixture was poured in a brass cast and samples obtained from the cast were rubbed and polished to have a close fit in the waveguide. The samples were desiccated[9] for at least 36 hr before the experiment.

Results and discussion

Table 1 gives the dielectric constants of the mixtures containing different proportions of crystal particles of six alkali halides in paraffin along with the values of u and x, as found from the graphs. The dielectric constants of crystals, determined by this method, are given in Table 2 along with the values obtained by von Hippel[10] for comparison. A good agreement has been found between the authors' values and those of von Hippel, showing the applicability of the method used. The slightly large difference in the two values arises from the fact that u values are determined graphically and a slight variation in u gives a large error in the value of ϵ_1. Though the method of calculation is a graphical one, the error has not increased beyond 1 per cent.

The dielectric constants of different alkali halides given in Table 3 show that the dielectric constants of sodium halides are greater than those of potassium halides and also dielectric constants of these halides show a regularly increasing trend from chloride via bromide to iodide. This behaviour can be explained if we consider the ionic polarization of these crystals. The dielectric constant of a material can be attributed to polarizability and may be due to electronic, ionic and orientation factors. The electronic contribution arises from the displacement of electrons in an atom relative to the nucleus, that is from the deformation of the electronic shell about the nucleus. The ionic or atomic contribution is due to the displacement oı

TABLE 1 — DIELECTRIC CONSTANTS OF MIXTURES

CONC. OF SALT %	KCl	KBr	KI	NaCl	NaBr	NaI
5	2·303	2·305	2·307	2·325	2·328	2·334
10	2·398	2·404	2·408	2·444	2·450	2·465
15	2·497	2·501	2·513	2·568	2·571	2·599
20	2·598	2·605	2·616	2·698	2·713	2·748
30	2·810	2·823	2·840	2·965	3·001	3·058
40	3·036	3·054	—	—	3·307	3·406
u	1·900	1·750	1·580	1·650	1·550	1·400
x	0·285	0·305	0·335	0·385	0·408	0·459

TABLE 2 — DIELECTRIC CONSTANTS OF CRYSTALS

CRYSTAL	AUTHORS' VALUES	VON HIPPEL'S VALUES	VALUES FROM INTERNATIONAL CRITICAL TABLES
NaCl	5·86	5·90	5·90
NaBr	6·10	—	—
NaI	6·72	—	—
KCl	4·77	—	5·03
KBr	4·88	4·90	5·10
KI	5·09	—	5·40

TABLE 3 — COMPARISON OF THE VALUES OF S

CRYSTAL	ϵ	n^2	$\Delta\epsilon$	LATTICE SIDE (a) Å.	$\omega_T \times 10^{-9}$	S VALUES	
						Authors'	Szigeti's
NaCl	5·86	2·25	3·61	5·63	4910	0·76	0·74
NaBr	6·10	2·62	3·48	5·96	4017	0·70	0·69
NaI	6·72	2·92	3·80	6·46	3508	0·72	0·71
KCl	4·77	2·13	2·64	6·28	4243	0·81	0·80
KBr	4·88	2·33	2·55	6·59	3397	0·78	0·76
KI	5·09	2·69	2·40	7·05	2941	0·71	0·69

deformation of a charged ion with respect to the other ions. The orientation or dipolar polarization arises when the substance is built up of molecules possessing permanent electric dipole moments. In the case of ordinary ionic crystals there is no dipolar contribution and ionic contribution is seldom larger than the electronic contribution.

The ionic contribution can be separated from the electronic contribution in the following manner. In the optical range of frequencies, the dielectric constant of a crystal arises almost entirely from the electronic polarizability so that

$$\epsilon = n^2 \quad \ldots\ldots\ldots\ldots \quad (5)$$

n being the refractive index of crystal in the optical range, while in the electrical frequency range, the dielectric constant arises from both ionic and electronic polarizabilities. By subtracting n^2 from ϵ, $\Delta\epsilon$, the contribution due to ionic polarizability is determined. These are given in Table 3 along with n, the refractive index.

Born[11] applied the displacement theory to ionic crystals placed in electric fields and obtained the relation for the contribution due to ionic polarizability:

$$\Delta\epsilon = \frac{2\pi e^2}{\omega_T^2 a^3}\left(\frac{1}{M}+\frac{1}{m}\right) \quad \ldots\ldots\ldots \quad (6)$$

where a is the side of the cubical shell, e the electronic charge, ω_T the absorption frequency (usually in the infrared region), M the mass of positive ions and m the mass of negative ions.

Equation (6) was derived by Born assuming that: (i) the forces are central, (ii) field is static, and (iii) ions are deformable but do not overlap.

In real crystals, however, the ions always overlap to some extent under the influence of electromagnetic field. Szigeti[12] has considered two types of forces: (i) long range and (ii) short range forces. He assumed that the Lorentz theorem is not valid and there is a field acting on each ion due to its neighbours even in a uniformly polarized sphere. He put

$$\mu = ZSe(x_+ - x_-) \quad \ldots\ldots\ldots \quad (7)$$

where μ is electric moment due to the ions, Z the mass number, and x_+ and x_- changed co-ordinates of ions. S is a correction factor for deviation from ideal heteropolar behaviour and depends on the short range dipolar interaction, which includes the overlapping behaviour of the ions. With these assumptions Szigeti obtained the following equation:

$$\Delta\epsilon = \left(\frac{n^2+2}{3}\right)^2 . S \frac{2\pi e^2}{\omega_T^2 a^3}\left(\frac{1}{M}+\frac{1}{m}\right) \quad \ldots \quad (8)$$

Equation (8) contains two correction factors not present in Born's equation and both take account of the electronic contribution to the polarization of the electromagnetic waves near the infrared region. S represents the short range interaction of electronic and atomic displacements. The factor $\left(\frac{n^2+2}{3}\right)^2$ appears because of the long range interaction of transverse nature of electromagnetic waves. The necessity for correction for the short range interaction has also been pointed out by Heckmann[13], Eucken and Buchner[14], Mott and Littleton[15] and others. In anisotropic crystals, however, S is not equal to unity even in an ideal heteropolar material, since the short range dipolar interaction does not vanish in such crystals even if there is no overlap.

The calculated values of S employing equation (8) are given in Table 3 along with Szigeti's values calculated from the results of Mott and Gurney[16]. A fair agreement in the values is observed. The authors' values are slightly higher than those of Szigeti showing that there is less overlap or short range interaction in the microwave region.

Acknowledgement

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X-ray Studies of High Temperature Transformations of South Arcot Lignite Ash

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X-ray studies of minerals in South Arcot lignite (ash content $<$8 per cent) have shown the presence of quartz and calcite, probably goethite, gypsum, felspar and haematite, all except quartz occurring in a very finely divided state. The ash obtained at 400° and 800°C. shows anhydrite as the main phase. One high iron, low-melting ash (m.p. $\sim$1150°C.) on heating to 1000°C., has shown the presence of anhydrite, magnetite and haematite with two lines at 10 A. and 3 A., attributable to calcium silicate hydrate (I). After heating to 1200°C. the anhydrite pattern is unobservable but lines due to gehlenite, periclase, magnetite, olivine and bands ascribable to calcium silicate hydrate (II) are present. Low iron, high-fusion ash, heated to 1300°C., very rapidly cooled and partially hydrated, shows the presence of anhydrite, $6CaO.4Al_2O_3.(Mg, Fe)O.SiO_2$, $3CaO.Al_2O_3.8$-$12H_2O$, $4CaO.Al_2O_3.Fe_2O_3$, α and γ-$2CaO.SiO_2$, $MgO.Al_2O_3$ and $Ca(OH)_2$. After 16 hr immersion in water, the 'quenched' ash shows the following mineral assemblage: anhydrite, ettringite, $3CaO.Al_2O_3$, $3CaSO_4.32H_2O$, γ-$2CaO.SiO_2$, $MgO.Al_2O_3$, ill-crystallized mixed calcium silicate hydrates and $Ca(OH)_2$.

The bearing of these results on the utilization of South Arcot lignite ash from thermal power plants is discussed and it is pointed out that as in the case of the expansive cements, the quenched ash mixed with Portland cement and blast furnace slag, in the right proportions, can give a suitable expansive cement.

THE present work was undertaken as part of a general programme of investigations on coal ash and its high temperature transformations since the understanding thus derived can help in the best utilization of ash from pulverized fuel furnaces, slagging ash generators, etc. Work on ash fusibility, in various atmospheres and for various compositions, has been carried out at this Institute and its Survey Stations; one of the desired ends in view, namely the prediction of fusion temperature and viscosity of the molten ash, is a complex problem, determined by many variables, some of them being better understood as a result of the analytical work carried out so far.

In a report[1] recently prepared by this Institute on the inorganic constituents of South Arcot lignite and the relationship between ash fusion and composition, it has been observed that the fusion temperatures of the overall samples of lignite ash in a mildly reducing atmosphere generally lie in the range 1110-1270°C., the low range being due to the high lime and low alumina and silica contents. It has been pointed out on the basis of chemical analyses and the fusion temperatures of ash from various size fractions that the silica content registers a maximum (83 per cent) for the $-0{\cdot}21$ $+0{\cdot}15$ mm. size fraction and decreases above and below this size range. Further, the Fe_2O_3, Al_2O_3, CaO, MgO and SO_3 contents are minimum for this size range. Also, the mineral matter of the lignite as determined by chemical analyses consists mainly of kaolinite, gypsum, quartz and hydrated oxides of aluminium and iron with minor traces of iron pyrites. It has been stated in the report that many of the samples, except those having a high fusion range ($>$1400°C.), melted to give a clear glass[1]. This observation as well as the high viscosity of the molten ash, specially in a mildly reducing atmosphere, has led to the present application of the X-ray method of analysis in understanding the high temperature transformations. Samples of ash, one representative of a low melting range (1100-1270°C.) and one of a high melting range ($>$1300°C.), have been studied so far.

Experimental procedure

Since the ash content of the overall samples of S.A. lignite does not exceed 8 per cent, the minerals

present in the lignite were studied by obtaining heavy fractions from distilled water suspensions of the powdered lignite within about a minute of adding water. These and the samples of ash heated to various temperatures were powdered finely and cylindrical specimens made with the help of dilute aqueous gum solution. The X-ray patterns were obtained using a Philips 11·46 cm. diam. camera, with the exit collimator replaced by a 6 mm. diam. lead cup. This helped considerably in detecting X-ray lines in the low angle region. Some of the ash samples at high temperature were powdered and kept under distilled water for various periods and then X-rayed to investigate the hydrated products. The films were processed under standard conditions and the *d* values determined measuring the Bragg angles with the help of a Philips distance measuring instrument. The identification of the crystalline components was carried out with the help of the A.S.T.M. Card Index and from published X-ray data.

Results and discussion

The compositions of the ash from overall samples of S.A. lignite (1) and S.A. lignite (3) and their ash-fusion ranges are given in Table 1. The *d* values for the heavy fractions of the lignite samples (1) and (2), the latter being a high-fusion type as (3), are given in Table 2. The data for the ash of S.A. lignite (1) obtained at 400° and 800°C., with the 800°C. ash heated further to 1000° and 1200°C. in a mildly reducing atmosphere are also given. The last sample had fused at about 1150°C. and the molten mass cooled from 1200°C. to about room temperature in the Leitz heating microscope furnace to give a hard, chocolate-brown, slightly honeycombed glass. The X-ray pattern contained comparatively few lines, superimposed on the heavy background due to iron and the amorphous phase. However, the presence of a definite but faint halo at about 9·7 A. indicated the presence of a hydrated phase which had formed during the preparation of the specimen with aqueous gum solution. A broad and more prominent line at about 10 A. had also been observed in the photograph of the sample heated to 1000°C.

TABLE 1 — CHEMICAL COMPOSITION OF S.A. LIGNITE ASH

Oxides	Ash, %	
	S.A. lignite (1)	S.A. lignite (3)
SiO_2	8·16	14·59
Al_2O_3	15·15	15·96
Fe_2O_3	14·01	2·56
CaO	29·51	32·16
MgO	8·19	5·36
SO_3	24·79	29·37
TiO_2	Trace	Trace
P_2O_5	0·04	0·04
Alkalies	0·15	Trace
Ash-fusion range (mildly reducing atmosphere)	1140-1270°C.	>1300°C.

Since this could be one of the several calcium silicate hydrates or calcium aluminate hydrates whose parent substance was present in the specimens, an X-ray study of a 'quenched' sample was undertaken to investigate the phase. This sample of S.A. lignite ash, of a slightly different composition and higher fusion temperature (Table 1), has been intensively studied. The *d* values for two specimens are given in Table 3. The first specimen was made by mixing one drop of distilled water to the powder and obtaining a thick paste from which a cylindrical specimen was rolled, and which set hard in a short time. The second specimen was made by keeping the powder immersed in a large quantity of distilled water for about 16 hr. In this case, it was observed that a pale yellow gel-like substance had formed with a distinct boundary under the water. This gel, on draining off the excess water and drying at room temperature, yielded curve-up layers. A square cross-sectional piece of suitable dimensions was cut and used as the specimen. Another large sample from the same batch was mixed with water and kept in a 100 per cent humidity atmosphere for a fortnight resulting in a hard-set sample. The X-ray pattern of this sample closely resembled the pattern of the water-immersed specimen and hence the data for this specimen have not been included in Table 3.

The minerals identified in S.A. lignite (2) are: goethite (α-$Fe_2O_3.H_2O$), a kaolin group mineral, quartz, calcite, possibly gypsum, felspar and haematite. They occur in a finely divided form, specially goethite, which is most probably present in a gel form rather than as a crystalline substance. Al_2O_3 and SiO_2 in S.A. lignite (1) are probably present as gels, with the Ca present as calcium humates, since no other line except one (4·14 A.) was observable in the X-ray photograph consisting of bands. The amount of each of the identified minerals was very small even in the heaviest fractions and except for quartz and the kaolin group mineral only the strongest line of each was detectable against the heavy background and the amorphous scattering from the lignite. Hence the identification of all the minerals except quartz, the kaolin group mineral and calcite is subjected to some uncertainty.

The ash prepared at 400°C. from S.A. lignite (1) shows anhydrite as the main phase with haematite and calcite in very small amounts. The ash prepared at 800°C. gives an almost identical pattern with

TABLE 2 — X-RAY ANALYSIS DATA (*d* VALUES IN A.) OF S.A. LIGNITE SAMPLES AND OF THE ASH HEATED TO DIFFERENT TEMPERATURES

$CaSO_4$	S.A. LIGNITE (1)	S.A. LIGNITE (2)	ASH OF S.A. LIGNITE (1) HEATED TO 400°C.	800°C.	S.A. LIGNITE (1) HEATED TO 1000°C.	1200°C.
		w (halo) 7·15 (k)			w (halo) 10·1 (I)	w band vw 9·71 (II)
	w 4·14 g	w 4·19 (g)				
vvw 3·85		vvw, spotty 4·24 (q)				
		vw 3·89				
		vw 3·56 (k)				vw 3·67 (geh)
s 3·46			m 3·47 (a)	m 3·48 (a)	m 3·45 (a)	
		w+, spotty 3·34 (q)				
			vw 3·30	vw 3·30	vw 3·30	
		vw 3·20 (f)				vvw 3·33
vvw 3·09		vw 3·05 (c)	vvw 3·02 (c)		w 3·00 (I)	br, vw 3·04 [II, geh]
						vvw 2·94
m 2·83			w 2·85 (a)	w 2·84 (a)	w 2·82 (a, I)	w+ 2·85 [geh, s II]
						vvw 2·76 (oli)
		vvw 2·70 (h)	vw, sh 2·69 (h)	vw, oh 2·70 (h)	vw 2·68 (h)	vvw 2·63
vw 2·46			w 2·51 (h)	vw 2·50 (h)	w 2·51 (m, h)	w 2·51 (m, oli)
					w 2·44 γ (m)	band ↑ 2·44 (geh, s, oli) ↓ 2·38 (geh)
m 2·31			vw 2·33 (a)	vw 2·32 (a)	vw 2·32a (γ)	w 2·27 (geh, oli)
m−2·20			w 2·21a	w 2·20 (a)	w 2·20 (a)	vw 2·18 (oli)
w+ 2·071			w 2·09 (a)	vw 2·08 (a)	w 2·08 (a)	w 2·11 (p)
w 2·006			vw 2·00 (a)	vvw 2·00 (a)		vw 2·03 (s, geh)
vvw 1·92				vvw 1·93		vw 1·93 (geh)
m 1·86			w 1·88 (a)	w, oh 1·87 (a)	w 1·86 (a)	vvw 1·84 (geh)
		vvw 1·81 (q)		vvw 1·84 (h)		vw 1·80 (II)
m−1·74			vw 1·76 (a)	vw 1·75 (a)	vw 1·74 (a)	w 1·75 (geh, oli)
			vvw 1·71 (h)	vvw 1·69 (a)	vw 1·69 (h)	
			vw 1·66 (a)	vw 1·65 (a)	vw 1·64 (a)	
m−1·64			vvw 1·61 (h)	vvw 1·60 (h)	vvw 1·60 (h, m)	vvw 1·62
vvw 1·59					vvw 1·59 (a)	vvw 1·60
w 1·56					vvw 1·53 (a)	vvw 1·55 (s)
vw 1·52			vw 1·50 (h, a)	vw 1·49 (h, a)	vw 1·49 (h, m, a)	vw 1·50 (p, oli)
w 1·49				vw 1·46h	vvw 1·46h	vvw 1·48 (m, oli)
vw 1·42			vvw 1·33 (h, a)		vvw 1·33 (h, a)	
vw 1·39						
w+ 1·32			vvw 1·29 (h)	vw 1·28 (h, a)	vw 1·29 (h, m, a)	
w++ 1·273						
vw 1·213				vvw 1·26 (h)		
vw 1·198				vw 1·22 (g, h)		
w++ 1·162				vw 1·17 (a, h)	vw 1·17	

(g), goethite (α-$Fe_2O_3.H_2O$); (k), kaolin group mineral; (q) quartz; (f) felspar; (c), calcite; (h), haematite; (oli), olivine, (Mg, Fe)SiO_4 (A.S.T.M. 2-1346); (a), anhydrite ($CaSO_4$); (m), magnetite (Fe_3O_4); (geh), gehlenite (2CaO, $Al_2O_3.SiO_2$); (s), (Mg, Fe)$O.Al_2O_3$-spinel; (γ), γ-Al_2O_3; (I), calcium silicate hydrate I; (II), calcium silicate hydrate II; (p), periclase (MgO). s, strong; m, medium; w, weak; vw, very weak; vvw, very very weak.

sharper lines but with the absence of the strongest line of calcite which decomposes at about this temperature. The identification of anhydrite, $CaSO_4$, as the main crystalline phase in the ash prepared at 400° and 800°C. is supported by chemical analyses. The *d* values and visually estimated intensities of an A.R. grade $CaSO_4$ sample were experimentally obtained and are given in Table 2, along with the *d* values obtained for the ash. Other possible substances with the strongest line at about 3·50 to 3·48 A., such as anhydrous $Al_2(SO_4)_3$ and $MgSO_4$, are ruled out due to their rapid hydration in water; many others based on hexagonal silicon-oxygen layers such as the chlorites, talc, amesite or structures based on three-dimensional silicon-oxygen tetrahedral networks such as zeolites (Mordenite, Thomsonite) or Meionite, Prehenite, etc., are ruled out, both on the grounds of the impossibility of their formation during ashing of the lignite and on the incompatibility of their other prominent lines with those of the ash. Other probable compounds such as $3CaO.5Al_2O_3$ or $CaO.2Al_2O_3$ require much higher temperatures for their formation.

The ash sample heated to 1000°C. shows a broad, halo-like line at about 10 A. and another of the same

TABLE 3 — X-RAY DATA (*d* VALUES IN A.) FOR THE S.A. LIGNITE (3) ASH, HEATED TO 1300°C.

S.A. LIGNITE (3) ASH, HEATED TO 1300°C., 'QUENCHED', PARTIALLY HYDRATED	S.A. LIGNITE (3) ASH, HEATED TO 1300°C., 'QUENCHED', AFTER 16 HR IMMERSION IN WATER	$4CaO.Al_2O_3.Fe_2O_3$ A.S.T.M. 2-0965	$C_3A, 3CaSO_4.31H_2O$*	ETTRINGITE (ART.) A.S.T.M. 2-0059	$CaSO_4$	$Ca(OH)_2$ A.S.T.M. 4-0733	$C_3A, 8\text{-}12H_2O$ A.S.T.M. 2-0083	THAUMASITE A.S.T.M. 2-0061	ILL-CRYSTALLIZED MIXED CALCIUM SILICATE HYDRATES
band, w 9·7	w 12·5		s 9·3	9·8 (100)				9·67 (100)	vs 12·5
	s 9·89								
band, w 7·46	vw 7·3		w 7·23				(100) 7·65	7·06 (70)	
	vw 6·11							6·11 (50)	
	m 5·55		m 5·66	5·7 (80)				5·50 (80)	vvw 5·3
	vw 4·97		w 5·02	4·9 (60)				5·20 (20)	
	vw 4·93					(74) 4·90			
band, w 4·67	m 4·64		w 4·68	4·67 (70)				4·83 (50)	
w 4·20	vw 4·22		vw 4·26	4·34 (20)			(20) 4·28	4·54 (70)	
vw 4·03									
w 3·83 (al)	m 3·84		m 3·92	3·87 (80)	vvw 3·85				
s 3·71				3·60 (30)			(90) 3·77	3·76 (80)	
							(20) 3·60		
s 3·48 (al)	s 3·47			3·45 (60)				3·49 (50)	
vw 3·29	m 3·27			3·26 (40)	s 3·46		(20) 3·40	3·39 (70)	
vw 3·22	vw 3·21								
vw 3·16									
vw 3·11					vvw 3·09	(23) 3·11		3·17 (50)	vs 3·07
w 3·05 (ga)	w+ 3·03		m 3·03	3·02 (30)			(40) 3·02		
m 2·85 (s, al)	w+ 2·84 (s)		m 2·81	2·79 (90)	m 2·83		(70) 2·86	2·92 (40)	s 2·80
w 2·74 (ga)	w 2·75	(80) 2·77		2·67 (30)			(40) 2·76		
s 2·67 (al)									
m+ 2·65	s 2·66	(100) 2·63				(100) 2·63		2·69 (80)	
	m 2·55	(20) 2·54	m 2·57	2·57 (80)				2·57 (60)	w/d 2·4
m 2·45 (s)	m 2·44 (s)	(20) 2·43		2·43 (30)	vw 2·46		(60) 2·46		

(s), spinel (Mg, Fe)$O.Al_2O_3$; (al), α-$2CaO.SiO_2$; (ga), γ-$2CaO.SiO_2$.

vs, very strong; s, strong; m, medium; w, weak; mw, medium weak; vw, very weak; vvw, very very weak; w/d, weak and diffuse.

*Data taken from *The Chemistry of Portland Cement* by R. H. Bogue (Reinhold Publishing Corp., New York), 1955, 753.

Figures indicated in parentheses in columns 3, 5, 7, 8 and 9 denote the relative intensities.

TABLE 3 — X-RAY DATA (*d* VALUES IN A.) FOR THE S.A. LIGNITE (3) ASH, HEATED TO 1300°C.— *Contd.*

S.A. LIGNITE (3) ASH, HEATED TO 1300°C., 'QUENCHED', PARTIALLY HYDRATED	S.A. LIGNITE (3) ASH, HEATED TO 1300°C., 'QUENCHED', AFTER 16 HR IMMERSION IN WATER	$4CaO.Al_2O_3.Fe_2O_3$ A.S.T.M. 2-0965	$C_3A, 3CaSO_4.31H_2O$*	ETTRINGITE (ART.) A.S.T.M. 2-0059	$CaSO_4$	$Ca(OH)_2$ A.S.T.M. 4-0733	$C_3A, 8\text{-}12H_2O$ A.S.T.M. 2-0083	THAUMASITE A.S.T.M. 2-0061	ILL-CRYSTALLIZED MIXED CALCIUM SILICATE HYDRATES
m 2·32	w 2·31			2·36 (10)	m 2·31		(60) 2·31	2·34 (40)	
band								2·27 (40)	
m+ 2·21 (al)	m+ 2·20	(60) 2·19	m 2·23	2·20 (80)	m 2·20		(20) 2·20		
band m+ 2·17	vvw 2·15	(60) 2·15	w 2·16	2·14 (60)				2·14 (80)	
	w 2·09						(50) 2·10		w/d 2·1
w 2·06	vw 2·06			2·06 (30)	w+ 2·07			2·09 (60)	
band									
m 2·05 (s)	w 2·03 (s)	(80) 2·03			w 2·01		(40) 2·00	2·01 (50)	
vw 1·98 (al)	vvw 1·98		vw 1·97						
	vvw 1·94			1·94 (30)		(42) 1·93	(40) 1·93	1·93 (50)	
m 1·91 (ga)	m 1·90	(100) 1·92		1·89 (20)	vvw 1·92			1·90 (50)	
m+ 1·87 (ga)	w+ 1·87	(60) 1·85			m 1·86		(40) 1·86	1·84 (30)	s 1·83
	w 1·84		w 1·85	1·84 (40)					
vw 1·80 (ga)	vw 1·80	(80) 1·81		1·80 (10)		(36) 1·80	(40) 1·82	1·80 (60)	
m 1·75 (al, ga)	vw 1·75	(60) 1·73		1·75 (40)	m 1·74			1·77 (10)	
	vw 1·73			1·70 (40)			(40) 1·70	1·73 (50)	
	vvw 1·68					(21) 1·69		1·69 (30)	
m 1·65	w 1·66		w 1·67	1·66 (60)	m 1·64		(70) 1·65		mw 1·67
	w 1·64							1·62 (60)	
m 1·62	vw 1·62			1·62 (20)	vvw 1·59		(20) 1·62	1·59 (40)	
m 1·57	w+ 1·57	(80) 1·57	vw 1·59	1·57 (40)	w 1·56				
m 1·56 (s)	w+ 1·56 (s)		w 1·55				(20) 1·50		
m 1·53	w 1·53	(80) 1·53		1·54 (20)	vw 1·52		(20) 1·46		
m 1·49	m 1·49	(60) 1·49		1·50 (40)	w 1·49		(50) 1·43	1·53 (50)	vw 1·53
m 1·43 (s)	m 1·45 (s)	(60) 1·45		1·45 (30)					

(s), spinel $(Mg, Fe)O.Al_2O_3$; (al), $\alpha\text{-}2CaO.SiO_2$; (ga), $\gamma\text{-}2CaO.SiO_2$.

vs, very strong; s, strong; m, medium; w, weak; mw, medium weak; vw, very weak; vvw, very very weak; w/d, weak and diffuse.

*Data taken from *The Chemistry of Portland Cement* by R. H. Bogue (Reinhold Publishing Corp., New York), 1955, 753.

Figures indicated in parentheses in columns 3, 5, 7, 8 and 9 denote the relative intensities.

intensity at 3 A. In addition to this, anhydrite lines are present with haematite, magnetite (and possibly γ-alumina) as identifiable phases. The X-ray pattern of the glass obtained at 1200°C. also showed bands corresponding to the large spacing and 3 A. line in addition to lines due to gehlenite ($2CaO.Al_2O_3.SiO_2$), MgO, olivine, Fe_3O_4 and spinel. The 10 A. line and 9·71 A. band along with the other lines and bands indicate the presence of calcium silicate hydrate (I)[2] and calcium silicate hydrate (II) respectively which along with the ill-crystallized mixed hydrates[3] are by far the most important hydrated components found in hydrated and set cement.

South Arcot lignite (3) *ash* — The ash of S.A. lignite (3) was heated to high temperature but this sample failed to melt even at 1300°C., and in order to preserve the high temperature phases, very rapid air cooling of the sample was carried out. A drop of distilled water on a finely powdered sample of the quenched substance was sufficient to give visible hydrating effects, and hard-setting cylindrical specimens could be made without adding gum arabic solution. The *d* values for one such hydrated specimen are given in Table 3. The very large number of sharp lines denoting several well-crystallized phases, as contrasted with the comparatively few and diffuse lines present in the fused (1200°C.) S.A. lignite (1) ash, brings out remarkably the effect of composition and quenching on the final phase assemblage. One interesting point to be observed here is the presence of two very strong lines at 3·72 and 3·48 A. Though at first the two strongest lines were regarded as due to one phase only, a thorough search of available literature and the A.S.T.M. Index showed that apart from the possible exception of petalite, α-$LiO_2.Al_2O_3.8SiO_2$, no single phase could satisfactorily explain the lines. However, the presence of petalite is ruled out since after immersion of the sample in water for about 16 hr (Table 3), the 3·72 A. line completely disappeared leaving the 3·48 A. line with a slightly reduced intensity. Moreover, the chemical analyses also preclude the presence of this phase. On the other hand, the 3·48 A. line together with several others fitted the $CaSO_4$ pattern well, leading to the conclusion that in the case of S.A. lignite (3) ash, a large part of the anhydrite phase has withstood the heating up to 1300°C. without decomposition or solution in a glass unlike in the case of the S.A. lignite (1) ash, which melted on heating to 1150°C. only. The fact that anhydrite can be heated up to high temperatures of the order of 1300°C. without complete decomposition is borne out by its high melting point, 1350-75°C., and by the chemical and X-ray analyses carried out on expansive cement clinker made on an industrial scale in France[4]. The X-ray analyses of a rapidly cooled sulpho-aluminate clinker (starting materials: gypsum, 50; red bauxite, 25; and chalk, 25 per cent; clinker analysis: CaO, 41·3; SO_3, 22·1; Al_2O_3, 19·9; SiO_2, 7·0; and Fe_2O_3, 5·7 per cent) revealed the presence of significant amounts of free anhydrous $CaSO_4$, calcium aluminate (particularly $5CaO.3Al_2O_3$), and calcium silicate, γ-$2CaO.SiO_2$[4].

In the present case, it has been concluded that $CaSO_4$ is the main phase in spite of the strongest line of $3CaO.5Al_2O_3$ or $CaO.2Al_2O_3$ also being at 3·5 A., because the latter hydrate in water and their other strong lines at 2·60 A. (A.S.T.M. Card No. 1-0572), 2·59 and 3·09 A. (A.S.T.M. Card No. 2-0392) for $3CaO.5Al_2O_3$, and 2·61 and 4·44 A. for $CaO.2Al_2O_3$ respectively are not present. Anhydrite, on the other hand, is hardly affected by water apart from its slight solubility and any decrease in its quantity after prolonged immersion in water is due to causes other than hydration, as discussed below.

The identification of the phase with the strongest line at 3·72 A. posed difficulties since this line along with several others (columns 1 and 2, Table 3) is drastically affected by immersion of the sample in water for about 16 hr. After elimination of very unlikely substances, for example the artificial ultramarines, and noselite, hâuynite, which require a much higher amount of Na_2O than present in the sample (basic structure: three-dimensional Si-O tetrahedral network), the hexagonal form of anorthite ($CaO.Al_2O_3.2SiO_2$) formed after heating the synthetic compound to about 2000°C. in an atmosphere of N_2, and of orthorhombic sulphur (A.S.T.M. Card 2-0324) with lines at 3·74, 3·15 and 2·94 A. (relative intensities 100, 90 and 70 respectively), the following four substances remain for consideration:

(1) 'Unstable $5CaO.3Al_2O_3$' [strongest lines at 3·75, 2·89 and 2·77 A.][5]
(2) $3CaO.Al_2O_3.8\text{-}12H_2O$
(3) $CaCO_3.CaSiO_3.CaSO_4.15H_2O$ (Thaumasite)
(4) $3CaO.2SiO_2$ (Rankinite) [strongest lines (A.): 3·77 (s), 3·14 (vs), 2·69 (vs), 2·56 (s), 1·799 (s)][6]

Of these, (1) has been recognized as a primary phase of crystallization in the quaternary system CaO-Al_2O_3-MgO-SiO_2 with the probable composition $6CaO.4Al_2O_3.MgO.SiO_2$[7]. Its FeO analogue has similar X-ray and crystallographic data and the mineral, as it occurs in aluminous cement, may be a solid solution of the two. The *d* values and intensities are affected by the replacement of MgO by FeO, but a literature survey failed to give recent data on the two homologues; the strongest lines of an iron-rich member are indicated above in square brackets. The unstable

$5CaO.3Al_2O_3$ reacts with water comparatively slowly with the initial and final sets occurring between 7 and 16 hr after mixing, and yielding a product with about 1500 lb./sq. in. crushing strength at the end of one day.

The *d* values for the second possibility, viz. $3CaO.Al_2O_3.8\text{-}12H_2O$, have been given in Table 3. Substances like $3CaO.Al_2O_3$ and $4CaO.Al_2O_3.Fe_2O_3$ react rapidly with water to produce the hexagonal $3CaO.Al_2O_3.6H_2O$, followed by the cubic form of the compound. However, on adding 30 per cent of gypsum to a $3CaO.Al_2O_3$ preparation, the hexagonal plates of calcium aluminate hydrate transformed to the cubic form with subsequent precipitation of calcium sulpho-aluminate followed by the reappearance of the hexagonal calcium aluminate hydrate which altered to the cubic $3CaO.Al_2O_3.6H_2O$[8]. The addition of 20 per cent gypsum to $4CaO.Al_2O_3.Fe_2O_3$ resulted in similar reactions which were slower. In the present case, the effect of adding a very small quantity of distilled water to the quenched sample containing $CaSO_4$, whose solubility is only slightly less than that of gypsum, seems to be to produce a hydrate agreeing more or less with the quoted hexagonal phase card values, except for the 7·65 A. line which is replaced on the film by a weak broad band.

The third possibility is the presence of Thaumasite the *d* values for which have also been given in Table 3. The sample during its preparation with distilled water, and subsequently, can absorb atmospheric CO_2 resulting in the formation of Thaumasite. This is a soft mineral which hardens on exposure to air and can exist in a hydrogel form also. Thaumasite has been considered an adsorption product in which a hydrogel like $CaO.SiO_2.nH_2O$ has adsorbed calcium carbonate and sulphate[9].

Lastly, the presence of Rankinite, $3CaO.2SiO_2$, is also possible though its strongest line is at 3·14 A. and only a very weak line at 3·16 A. is present in the film and hence Rankinite cannot be the main component giving rise to a strong line at 3·72 A.

Since the pattern of ettringite, $3CaO.Al_2O_3.3CaSO_4.32H_2O$, becomes the most prominent one in the more completely hydrated samples, the crystallization of artificial ettringite requiring the hexagonal aluminate-hydrate, $CaSO_4$, and water[10], the presence of $6CaO.4Al_2O_3.(Mg, Fe)O.SiO_2$ together with $3CaO.Al_2O_3.8\text{-}12H_2O$ has been taken to give rise to a strong line at 3·72 A. The full pattern and its subsequent transformation on fuller hydration require more components than these two for an adequate explanation; from the *d* values and relative intensities coupled with information about their conditions of formation and properties specially with reference to water, the following have been assigned to be the components of the partially hydrated specimen apart from amorphous and ill-crystallized phases and those present in traces: $CaSO_4$, $6CaO.4Al_2O_3.(Mg, Fe)O. SiO_2$, $4CaO.Al_2O_3.Fe_2O_3$, $3CaO.Al_2O_3.8\text{-}12H_2O$, α-$2CaO.SiO_2$, γ-$2CaO.SiO_2$, $MgO.Al_2O_3$, $Ca(OH)_2$ and probably Thaumasite ($CaCO_3.CaSiO_3.CaSO_4.15H_2O$). The crystalline components of the more fully hydrated sample are as follows: $CaSO_4$, ettringite, $3CaO.Al_2O_3.3CaSO_4.32H_2O$, γ-$2CaO.SiO_2$, $MgO.Al_2O_3$, an ill-crystallized mixed hydrate with 12·5 A. for the 002 line, and $Ca(OH)_2$. The *d* values and intensities for many of the above crystalline components are variable within limits due to slight changes in composition and other factors. For instance α-$2CaO.SiO_2$, stable at high temperature, can only be crystallized in the presence of small amounts of stabilizers and the *d* value for the strong line at 2·83 A. has been found to vary from 2·80 to 2·86 A. and that for 2·68 A. from 2·65 to 2·70 A. with corresponding variations in other spacings[11].

From the X-ray results it thus appears possible that during the heating of the high-fusion ash at 1300°C. some of the $CaSO_4$ decomposes with the evolution of SO_3 and the lime set free together with the lime in excess of the original SO_3 content, i.e. about 12 per cent out of the 32·16 per cent (Table 1), reacts with the SiO_2, Al_2O_3 and the iron oxides to give the crystalline components given above some of which because of the ‘quenching’ remain in the unstable reactive forms.

As a result of these findings, the potential use of the quenched, high-fusion range ash from the proposed thermal power station at Neyveli assumes importance. Though it cannot replace cement in constructional work requiring rigid specifications of composition, strength and durability, its possible use for other building purposes or as sulpho-aluminate clinker to be used as the expansion agent in conjunction with Portland cement and blast furnace slag seems to be highly promising. Here it is worthwhile to refer again to Lafuma's paper[4] wherein, after pointing out the disadvantages of the shrinkage of cement on drying and of counteracting the shrinkage by using expansion agents like lime or magnesia or calcium sulpho-aluminate, he emphasized that the first expansion agent to give satisfactory practical results was a sulpho-aluminate clinker whose oxide composition has been given earlier in this paper, and which in terms of the actual substances present is composed of calcium sulphate, *c.* 38 per cent; α-$2CaO.SiO_2$, *c.* 20 per cent; aluminates, ferrites and alumino-ferrites, *c.* 38 per cent; and impurities, *c.* 4 per cent. He has given curves to show the beneficial effect

(as expansion per metre) after adding 8 to 20 per cent of the sulpho-aluminate clinker in suitable increments to Portland cement and also on the compressive strength which in two cases is even better than that of Portland cement alone. However, the use of such expansive cements is specially suitable when a rigid mould is used and aeration is kept to a minimum. Ettringite, whose crystallization is the main cause of the expansion, becomes converted in time to give the calcium aluminate, $4CaO.Al_2O_3.nH_2O$, and gypsum.

Much work on a large scale on tests for expansion and strength and on finding the uniformity of ash composition would have to be carried out if the S.A. lignite ash is to be used as an expansion agent in cement-blast furnace slag mixtures.

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A Rapid *in situ* Method for Determining Thermal Conductivities

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A simple *in situ* method for the rapid determination of thermal conductivities of wall and partition panels and other insulating and building materials is described. The transient heat flow principle is utilized and allowances are made for possible errors. The method gives results accurate to within 2-3 per cent of the accepted values which is the accuracy obtainable with conventional steady-state methods.

IN the development of suitable insulating and building materials for use in building construction, where there is a need to test a large number of samples, a quicker method than the conventional guarded hot plate method and which gives at the same time a comparable accuracy in the results for thermal conductivities, is preferable for general adoption. Transient radial heat flow methods, using an axial linear heat source, prove promising in the context. Vander Held andVan Drunen[1], Hooper and

Lepper[2] and others[3-5] have applied such a method for the determination of thermal conductivities of liquids and solids.

Theory

The basic mathematical treatment of transient heat flow in materials, applying different boundary conditions, has been given by Ingersoll *et al.*[6] and Carslaw and Jaeger[7].

The Fourier equation for heat conduction for a line source,

$$\frac{d\theta}{dt} = \alpha \left(\frac{d^2\theta}{dr^2} + \frac{1}{r} \cdot \frac{d\theta}{dr}\right) \quad \ldots\ldots (1)$$

when solved for the special case, where heat is flowing radially at a constant rate from an infinitely long line source embedded in an infinitely extending medium initially at zero temperature, gives θ to an acceptable degree of approximation as

$$\theta = \frac{q}{4\pi K}\ (\log_e t + C) \quad \ldots\ldots (2)$$

where K is the coefficient of thermal conductivity of the material surrounding the line source, q is the rate of heat release from unit length of the source, θ is its temperature at time t from start, and C is a constant. Measuring θ as a function of t and plotting it against $\log_e t$ will result in a straight line graph of slope $q/4\pi K$. It will be possible with this measured slope to calculate K when q is known. K is, however, determined with the help of the formula,

$$K = \frac{q}{4\pi(\theta_2 - \theta_1)} \cdot \log_e \frac{t_2}{t_1} \quad \ldots\ldots (3)$$

where θ_1 and θ_2 are the temperatures of the source at times t_1 and t_2 respectively.

Experimental procedure

The experimental arrangement consisting of a thermal probe and its associated electrical circuit is shown in Fig. 1. 34 s.w.g. constantan wire is stretched axially inside a thin copper tube, $\frac{1}{8}$ in. in outside diameter. A thermocouple junction D is placed in contact with the wire at its mid-point. A pointed steel conical plug termination at the free end facilitates easy introduction of the probe into the material. An ebonite handle is provided at the other end.

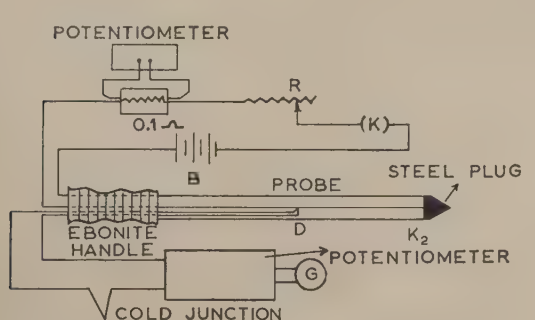

FIG. 1 — EXPERIMENTAL ARRANGEMENT FOR DETERMINATION OF THERMAL CONDUCTIVITY

The constantan wire is heated electrically, the heating current being supplied by two 6 V. heavy duty storage batteries in parallel, adjusted by the rheostat R and measured with a standard resistance and potentiometer. Another, but very sensitive, potentiometer is used to measure the thermal e.m.f. and hence the temperature at the mid-point of the line source.

The thermal probe is inserted easily when the material under test is a homogeneous loose fill like vermiculite, sand or soil. The probe can also be pushed in easily inside a cork slab. Where the material is a hard solid block, a hole of a suitable diameter can be initially drilled in it and the probe introduced thereafter as a tight fit. In a few cases, like concrete blocks, the probe can be cast along with the material itself.

After introducing the probe, sufficient time is allowed for it to attain the same temperature as the material, and then the current is switched on and the temperature rise at the middle is measured at different intervals. Curve I in Figs. 2-7 gives the temperature rise at different intervals in the case of the materials employed in this study.

Differentiating equation (2) with respect to time we get

$$\frac{d\theta}{dt} = \frac{q}{4\pi K} \cdot \frac{1}{t} \quad \ldots\ldots\ldots (4)$$

Plotting the reciprocal of $d\theta/dt$ against t should give a straight line curve passing through the origin. In actual practice this straight line cuts the X-axis at a point $t = t_o$ (curve II, Figs. 2-7). This lag should be ascribed to the initial time taken, on account of the finite diameter of the probe, before the heat flows into the surrounding conducting medium. But the slope of this curve is still $q/4\pi K$ which when measured will lead to a determination of K knowing q. Alternatively equation (3) can be modified as

$$K = \frac{q}{4\pi(\theta_2 - \theta_1)} \cdot \log_e \frac{t_2 - t_o}{t_1 - t_o} \quad \ldots (5)$$

where t_o is the correction to be applied for the finite diameter of the probe, which is obtained from the curve given by equation (4) (curve II, Figs. 2-7). $\text{Log}_e(t - t_c)$ is plotted against temperature rise in curve III (Figs. 2-7).

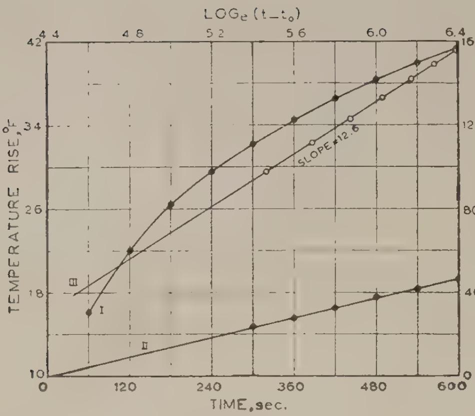

FIG. 2 — EXPERIMENTAL CURVES FOR CORK SLAB

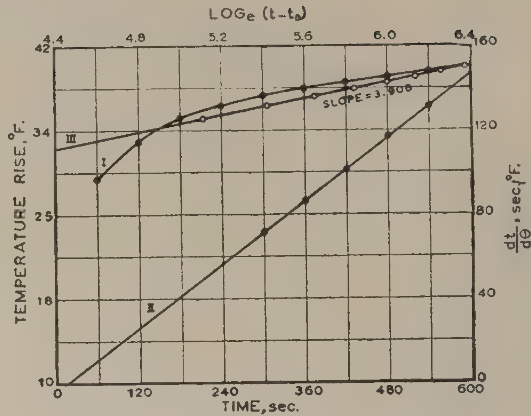

FIG. 5 — EXPERIMENTAL CURVES FOR SOIL

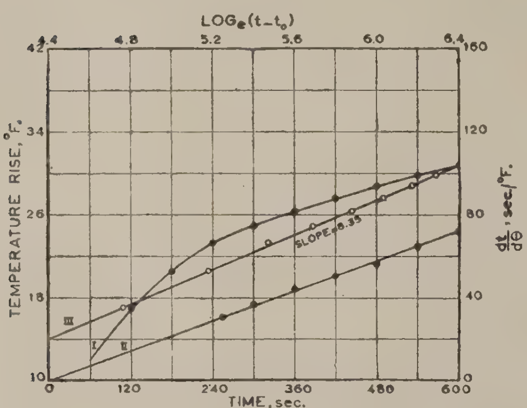

FIG. 3 — EXPERIMENTAL CURVES FOR FOAMED CONCRETE

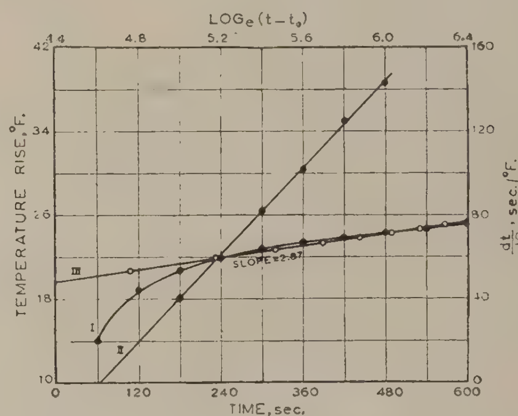

FIG. 6 — EXPERIMENTAL CURVES FOR SAND

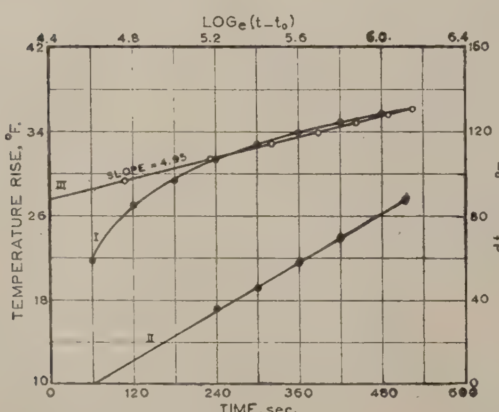

FIG. 4 — EXPERIMENTAL CURVES FOR EXFOLIATED VERMICULITE

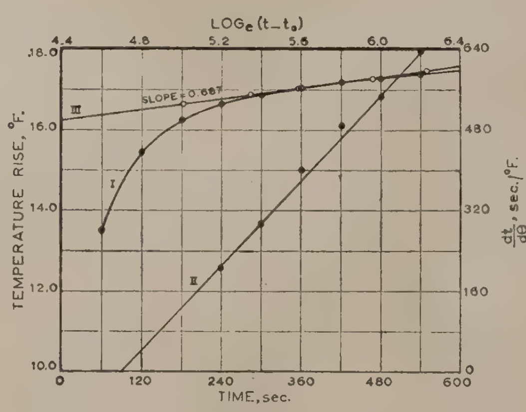

FIG. 7 — EXPERIMENTAL CURVES FOR BRICK WALL

The surrounding medium in the experimental set-up cannot extend to infinity as assumed in theory. However, the finite extent of the medium will have negligible effect on the accuracy of the results, provided readings are confined to the interval before the heat front has had time to reach the outer limits of the medium. A preliminary experiment employing thermocouples embedded at different radial distances from the probe showed that the heat front does not penetrate more than 3 in. in 10 min. inside any material similar to those taken up in these investigations. Accordingly, a minimum of 4 in. radial dimensions for the test material and a maximum of 10 min. time from start for taking readings have been adopted in these investigations to eliminate possible errors arising out of the finite extension of the medium.

Hooper and Lepper[2] suggested a minimum length-diameter ratio of 100:1 in order to ensure good accuracy in the results. Preliminary experiments conducted by the author also showed that consistent values were obtained if the length-diameter ratio was not less than 100: 1. For shorter lengths, the calculated values for K tended to increase. The values stabilized themselves at a constant value when the length-diameter ratio rises to 100: 1 and above. Accordingly, the thermal probes used in these investigations were designed to have length-to-diameter ratios equal to 100: 1.

Coefficients of conductivity were determined using the thermal probe for the following materials: cork, foamed concrete, vermiculite, sand, soil and a prototype wall. The thermal probe could be easily inserted into cork and foamed concrete. In the case of sand, vermiculite and soil, the material was contained in a cylinder about 8 in. in diameter or in a container of larger dimensions with rectangular or square cross-sections.

In situ determinations were also made in the case of a prototype brick wall. The laboratory wall was chosen. It was $22\frac{1}{2}$ in. thick, built with brick in mortar, about 7 years ago. A hole of sufficient length was bored through the wall using a high speed drill fitted with a tempered boring tool of sufficient length. The diameter of the bore made was just enough to ensure a tight fit for the probe. The bore in a few cases when finished was irregular and slightly larger than necessary. In such cases mortar of the same mix as that in the wall was used as a filler round the probe. The required quantity of the mortar when fresh was introduced into the bored hole, and before it set the probe was also introduced and pulled in and out and turned about, so that the hole was smoothened and reduced to the correct diameter to give a satisfactory contact between the probe and the wall.

TABLE 1 — THERMAL CONDUCTIVITIES OF DIFFERENT MATERIALS

Material	Bulk density *lb./cu. ft*	Thermal conductivity *B.t.u./(sq. ft) (hr) (°F. per in.)*	
		Guarded hot plate method	Thermal probe method
Cork slab	12·00	0·305	0·306
Foamed concrete	14·00	0·358	0·365
Exfoliated vermiculite	16·50	—	0·483
Dry soil (Moradabad)	83·66	—	2·070
Dry sand	95·00	—	2·030
Laboratory brick wall	—	—	5·620

Results and discussion

The curves obtained for the different materials are presented in Figs. 2-7. The values for the coefficients of thermal conductivity were calculated using the slope of the curve III (Figs. 2-7). The values are given in Table 1. The accepted values for the conductivities, where available, are also tabulated to show that the probe method gives results which are accurate to within about 2 to 3 per cent of the accepted values.

Acknowledgement

Thanks are due to Prof. T. N. Seshadri for valuable discussions and guidance and to Shri K. R. Rao for initiating the work. The paper is published with the permission of the Director, Central Building Research Institute, Roorkee.

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Decomposition of Carbon Tetrachloride in High Pressure Bombs

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The decomposition of carbon tetrachloride under different conditions of pressure (5-500 atm.) and temperature (200-400°C.) in a high pressure bomb has been investigated with a view to finding out whether the carbon-halogen bond in carbon tetrachloride can be broken up by heat and pressure alone. It has been observed that at 150 atm. and at 300°C., the main reaction product is hexachlorobenzene. A reaction mechanism involving the free radical $\cdot\dot{\underset{\cdot}{C}}Cl$ has been suggested.

IN an earlier communication by the author[1], the results of studies on the decomposition of carbon tetrachloride in aqueous solutions under the influence of ultrasonic waves were reported and it was shown that the decomposition proceeded faster at lower temperatures. The present investigation was undertaken with a view to finding out whether the carbon-halogen bond in carbon tetrachloride can be broken up by heat and pressure alone and the results are reported in this paper.

Experimental procedure

Doubly distilled carbon tetrachloride was used in all the experiments. By gas phase chromatographic analysis it was found to be 99·96 per cent pure and completely free from water vapour. To ascertain whether trace impurities present to the extent of 0·04 per cent have any influence on decomposition, carbon tetrachloride vapours were passed through a glass tube heated to 400°C. No decomposition was observed to take place.

The high pressure bomb used was made of low chromium-nickel-molybdenum steel, similar to one used by Bhattacharyya and Sourirajan[2], with the only difference that in the present investigation no copper lining was used.

In the preliminary experiments, carbon tetrachloride vapours were passed through a glass tube lined with the same alloy of which the bomb was made and heated to 300°C. A little decomposition was observed to take place under these conditions. To confirm this, experiments were repeated in sealed glass tubes containing carbon tetrachloride and the alloy or copper filings. Before sealing the tube, carbon tetrachloride was frozen using liquid air and the tube evacuated to ensure complete removal of air. A blank experiment, in which carbon tetrachloride alone was sealed within the glass tube, was also performed. The tube employed in the blank experiment and the one containing the alloy were kept at 300°C. for 1 hr, and the tube containing copper filings was kept at 125°C. for 1 hr. While decomposition resulting in the formation of chlorine and metal chlorides was observed in the tubes containing the alloy and copper filings, no decomposition was observed in the tube containing carbon tetrachloride alone. When carbon tetrachloride was used without redistillation or the tubes were not cleaned properly, slight decomposition was observed, which may be due to reaction occurring on the glass surface, as suggested by Schwarz and Pfhgmacher[3].

Bomb experiments — The following experiments were performed:

(i) Experiments were carried out in the bomb without any lining in an atmosphere of nitrogen. The reaction pressure was varied from 95 to 500 atm. and temperatures from 200° to 300°C.; carbon tetrachloride was kept in a steel side tube.

(ii) Experiments were carried out in the bomb without any lining in an atmosphere of carbon tetrachloride vapour formed by keeping the solvent in the bomb itself. The reaction pressure was varied from 100 to 200 atm. and temperature from 200° to 400°C.

(iii) Experiments were carried out in the bomb lined with glass so that the contact between carbon tetrachloride and the metal surface was minimum. The reaction was carried out in an atmosphere of carbon tetrachloride vapours formed by keeping the

solvent directly in the bomb. The reaction pressure varied from 5 to 150 atm. and temperature from 200° to 400°C.

Results

In all the three types of experiments carried out, the liberation of considerable amounts of chlorine was observed and a crust of solid was found deposited on the walls of the bomb. The solid contained, depending upon experimental conditions, varying proportions of carbon particles, chlorides of iron, nickel and chromium and organic compounds. No molybdenum was detected in the deposit.

In the bomb experiments, the amount of hexachlorobenzene formed was maximum when the reaction pressure was 150 atm. and temperature 300°C. Above 500 atm., carbon constituted the main product along with some chlorides of the metals forming the body of the bomb. At very low pressures, considerable amount of chlorine was liberated; chlorides of metals and some decomposed carbon tetrachloride were also detected. When the reaction was carried out at 150 atm. and 400°C., and in the presence of carbon tetrachloride vapour, an induction period was observed. There was a slight fall in temperature during the induction period and after the induction period was over, the temperature rose slightly, accompanied by fall in pressure from 150 to 50 atm. within 15 min.

Identification of products — The organic portion consisted of two products: a white crystalline substance and a brown substance. The white crystalline substance was found to consist of two fractions with different solubilities in acetone. The less soluble portion was identified as hexachlorobenzene from chemical analysis, physical properties and infrared analysis (carbon, 26·67 per cent; chlorine, 73·2 per cent; mol. wt. 308*, 282†; dipole moment, $< 0{\cdot}3$; density, 2·0; m.p. 228-9°C.; mixed m.p. with C_6Cl_6, 227-8°C.). From the infrared analysis curves, the more soluble fraction was also found to contain mostly hexachlorobenzene (carbon, 26·57 per cent; chlorine, 73·4 per cent; mol. wt, 235). The infrared spectrum of the freshly extracted compound showed the presence of an extra peak, which, however, disappeared after a month's storage. The extra peak was, therefore, probably due to the presence of a compound capable of subliming at room temperature.

The brown product, separated from the white crystalline product by sublimation under low pressure, had a characteristic camphor-like odour, which may be due to a little amount of hexachloroethane. It also contained small amounts of hexachlorobenzene; m.p. 100°C. Its infrared spectrum did not show any characteristic peak. At higher concentrations, the 1350 cm.$^{-1}$ peak of hexachlorobenzene was observed.

*Determined by Drs Strause and Weiler, London.
†Determined by the author.

Discussion

From the results of experiments carried out in sealed tubes it is clear that the decomposition of carbon tetrachloride is initiated and catalysed by the metal surface; copper used for the gasket of the bomb may also be playing a part[4]. The probable formation of the following radicals is also indicated: $\dot{C}Cl_3$, $Cl^\bullet$, $\cdot\dot{\underset{\bullet}{C}}\cdot$ and $\cdot\dot{\underset{\bullet}{C}}Cl$. Schwary and Pfhgmacher have made a similar suggestion to explain the formation of C_2Cl_6, C_6Cl_6 and certain other products during pyrolytic decomposition of carbon tetrachloride between 600° and 1100°C. A similar reaction between 700° and 1500°C. resulting in the formation of C_2Cl_6, C_2Cl_4, C_2Cl_2, C_6Cl_6 and C in the order of increasing temperature was reported by Regnault[5]. Some other workers[6,7] have also reported the formation of hexachlorobenzene from carbon tetrachloride. Bolton[8] observed the formation of hexachlorobenzene from carbon and chlorine in a carbon arc, which indicates that atomic carbon may first combine to form $\cdot\dot{\underset{\bullet}{C}}Cl$, which under pressure cyclizes to form hexachlorobenzene before it can be further chlorinated. At lower pressure, the same radical may give rise to C_2Cl_2, which on further chlorination may give C_2Cl_4 and C_2Cl_6. But, the synthesis of carbon tetrachloride from carbon and chlorine cannot be normally expected unless the C—C or the C=C bond is broken without the rupture of the C—Cl bond. This seems to be the probable reason for the observation reported by Fink and and Bouilla[9] that although the synthesis of carbon tetrachloride from carbon and chlorine is thermodynamically feasible, it cannot be achieved in practice to any appreciable extent, and on the contrary compounds such as C_2Cl_2, C_2Cl_4, C_2Cl_6 are obtained. The mechanism of the reaction appears to be complicated and is under investigation on the basis of kinetic data and spectroscopic evidence.

It is clear from the results of this study that the type of bomb employed in the present study or even glass vessels are not suited for carrying out this type of investigations, where the decomposition of bond only by temperature and pressure, unaffected by the wall of the reaction vessel, is aimed at to throw light on the theory of cavitation by ultrasonic waves.

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Kinetics of Coal Carbonization Reactions in the Plastic State

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The kinetics of coal carbonization reaction in the plastic state have been studied by isolating the metaplast produced by shock-treating coal and extracting it with chloroform. The activation energies of the above reaction for a good coking coal and a semi-coking coal have been determined. The study of the activation energies of the reaction in the case of a non-caking coal has yielded some information on the possible mechanism of coke formation.

COKING coal from the plastometric point of view has two constituents: fusible and non-fusible. The fusible part on carbonization passes through a fluid state into semi-coke while the non-fusible part directly passes from one solid phase to another. Some workers[1-5] visualize the following two consecutive reactions occurring during the former transformation:

$$\text{Solid coal (fusible)} \xrightarrow{(A)} \text{metaplast} \xrightarrow{(B)} \text{semicoke} + \text{volatiles}$$

The kinetics of these reactions have been studied by two different methods — thermogravimetric[2,3,6] and plastometric[5]. In the following investigation a more direct approach has been attempted by isolating the metaplast produced in reaction (A) and eliminated in reaction (B).

Reactions (A) and (B) have been proved to be of the first order[2-6]. Neglecting the contribution of volatiles, the concentration of the metaplast at a particular temperature can be shown to obey the following mathematical relations:

$$f = \frac{k_1 a}{k_1 - k_2}[e^{-k_2 t} - e^{-k_1 t}] \quad \ldots\ldots \quad (1)$$

where f is the concentration of fluid coal, k_1 and k_2 the velocity constants of reactions (A) and (B) respectively, t, the period for which the coals were heated and a, initial concentration of the fusible coal, which is constant for a particular system. Taking logarithm and differentiating,

$$\frac{dlnf}{dt} = -k_2 + \frac{k_1 - k_2}{e^{(k_1-k_2)t} - 1} \quad \ldots\ldots \quad (2)$$

The experimental plot of log f versus t (Fig. 1) is a straight line which passes through a maximum and then has a negative slope. The appearance of maximum indicates that $k_1 > k_2$. For sufficiently large values of t, equation (2) reduces to

$$dlnf = -k_2 \quad \text{or} \quad \frac{d \log f}{dt} = \frac{-k_2}{2{\cdot}3} \quad \ldots\ldots \quad (3)$$

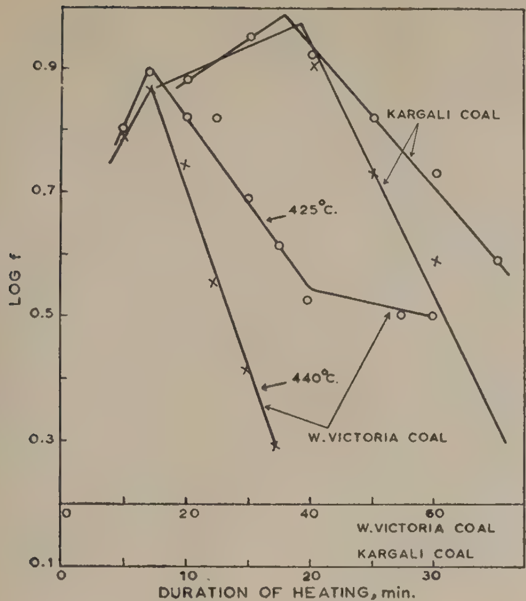

FIG. 1 — RELATION BETWEEN LOG f AND DURATION OF HEATING IN THE CASE OF KARGALI AND WEST VICTORIA COALS

That is, the slope of the linear portion of the figures by 2·3 gives the velocity of reaction (B) at the temperature of the experiment.

Temperature dependence of reaction velocity constant is given by the Arrhenius equation:

$$k = k_o e^{-E/RT}$$

where k_o is the frequency factor, E the energy of activation, R the gas constant and k the reaction velocity constant at the absolute temperature T.

Taking logarithm of the terms on both sides of the equation:

$$ln k = ln k_o - \frac{E}{RT} \text{ or } \log k = \log k_o - \frac{E}{2{\cdot}3RT} \quad \ldots \quad (4)$$

Thus, velocity constants k' and k'' corresponding to temperatures T_1 and T_2 respectively are related by

$$ln \frac{k'}{k''} = \frac{E}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

$$\text{or } \log \frac{k'}{k''} = \frac{E}{2{\cdot}3R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \quad \ldots\ldots \quad (5)$$

Equations (4) and (5) enable calculation of both k_o and E for the reaction (B).

It is known that preheating of coal enhances its solubility[7-9]. This is in accordance with the depolymerization concept of reaction (A). It is, therefore, considered that the extract of coal, in a suitable solvent, may be used as the concentration of metaplast in the system. Chloroform, which has been advocated by Dryden and Pankhurst[10] as a suitable solvent for preheated coals, has been used in the present work.

In the present experiment the two coal samples have been heated at two isothermal temperatures of 425° and 440°C. respectively for different lengths of time and then quickly chilled to arrest the reactions. The metaplasts thus prepared have been extracted with chloroform in a soxhlet extractor for about 48 hr till the effluent is colourless. The solvent was distilled off, the final traces being removed under suction.

One of the coals, a good coking coal from the Kargali seam of Swang colliery (East Bokaro coalfield) had the proximate analysis: V.M., 34·8 and F.C., 65·2 per cent (d.m.m.f. basis). The other, a semi-coking coal from the Laikdih seam of West Victoria colliery (Raniganj coalfield), had the proximate analysis: V.M., 29·9 and F.C., 70·1 per cent (d.m.m.f. basis).

The period for which the coals were heated at 425° and 440°C. have been plotted against the logarithm of the percentage extract (log f) in the case of Kargali and West Victoria coals respectively (Fig. 1). The values of k_2, k_o and E calculated from the slopes of the graphs in Fig. 1 are given below:

Kargali coal

(% chloroform extract of the original coal = 1·40)

Slope	
at 425°	0·023
at 440°	0·040
k_2	
at 425°	0·053 min.$^{-1}$
at 440°	0·092 min.$^{-1}$
k_o	2×10^{12}
E	37 k.cal.

West Victoria coal

(% chloroform extract of the original coal = 1·43)

Slope	
at 425°	0·015
at 440°	0·029
k_2	
at 425°	0·034 min.$^{-1}$
at 440°	0·065 min.$^{-1}$
k_o	$1{\cdot}2 \times 10^{12}$
E	42·6 k.cal.

The values of E and k_o obtained in the present work are somewhat lower than those obtained by earlier workers[11] who reported E around 48 k.cal. and k_o of the order of 10^{14} for good coking coals. In the case of semi-coking coal, the frequency factor (k_o) is a bit lower and the energy of activation (E) somewhat higher than those in the case of good coking coals.

The present work has been extended to the study of a non-coking Jambad Bowlah coal from the Raniganj field. The percentage of chloroform extract is very small and does not allow calculation of k_o and E. The percentage extract of the original coal and of coal heated at different temperatures and for different intervals are given below.

Jambad Bowlah coal (chloroform extract, 1·18%)

TEMP. °C.	CHLOROFORM EXTRACT (%) OF COAL HEATED FOR		
	10 min.	15 min.	20 min.
425	0·62	0·35	0·17
385	1·70	0·95	—
365	1·60	1·19	0·85

Earlier work[2-6] on the rate processes of coal carbonization suggests that the chemical reactions involved in the carbonization of coking, semi-coking and non-coking coals are similar in nature. On heating, the complex molecules of coal are degraded into simpler ones which reunite to form again some complex molecules and undergo further transformation at higher temperatures. Throughout these processes coal loses its weight due to the escape of the volatile products of decomposition. Depending upon the exact chemical constitution and physical structure, the life of the simple molecules of degradation, and hence the fluid behaviour induced by them, vary widely. Due chiefly to the preponderance of reactive groups, low rank, non-coking coals produce, on heating, highly reactive molecules possessing a transient life and leading to the absence of plastic state and hence of coking property. The present work on the isolation of metaplast lends direct support to the above view. It is to be noted that small amount of extract with chloroform is insufficient to plasticize the immobile, cross-linked structure rapidly produced by the transient molecules. Further, it has been observed by the present authors almost simultaneously with Oxley and Pitt[12] that chloroform extract persists beyond the point of resolidification in the case of coking and semi-coking coals. The chloroform extract from a coal heated at any temperature (i.e. in the temperature range of formation of metaplast) for any period of time, if at all is obtained, does melt on heating at about 300°C. Therefore, it seems reasonable to assume that the absence of fluid behaviour after a certain period of time is due to the inability of the extract to plasticize the semi-coke already produced. Oxley and Pitt[12], on the other hand, believed that there is a change in quality and nature of the metaplast or the ‘ extract ’ as they term it, which is formed beyond the resolidification temperature. The melting behaviour of the metaplast when obtained appears to contradict this view.

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Observations on the Association of Acetyl Group with Components of Jute

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A method has been described for extracting the major portion of hemicellulose from the isolated holocellulose with water under pressure. Analyses of the crude and purified products indicate that the acetyl group constitutes a characteristic functional group of hemicellulosic components, although a small amount seems to be in combination with alpha-cellulose. Of the different components of hemicellulose, hexosans, which have been found to comprise galactose, glucose and traces of mannose, account for only a small fraction of acetyl, while the group appears to be mostly in association with either xylose or uronic acid residue or both.

THE presence of acetyl group in jute has been demonstrated by a number of workers[1-3], and it has recently been shown[4,5] that the acetyl content affords a satisfactory means of distinguishing between the two botanical species of jute fibre (white and tossa) as well as between jute and its substitute fibres, such as bimli, mesta and kenaf.

Since the major portion of acetyl group occurring in jute can be traced in the isolated holocellulosic fraction[3], it is likely that this group exists mainly in combination with carbohydrate components. The small loss of acetyl group (10-15 per cent) occurred during delignification[3,6] but it cannot be taken as an evidence for its association with lignin, as there is a distinct possibility of this portion being attached with such carbohydrate units so as to be easily removed by the action of the delignifying reagent.

The carbohydrate fraction of a plant substance including jute fibre is known to be composed mainly of true or alpha-cellulose and hemicellulose; it is the latter fraction with which the acetyl group is believed to be associated, obviously through an ester linkage, since it is easily hydrolysed by the action of alkali. The usual method of isolation of hemicellulose, which consists in extracting the delignified material, preferably holocellulose, with strong alkali, readily effects the hydrolysis of this ester, and the isolated hemicellulose is practically free from acetyl group. In the case of certain ligno-cellulosic substances[7,8], however, isolation of hemicellulose containing a certain amount of acetyl group has been made possible by extracting the delignified material with boiling water instead of alkali. Because of the complication arising out of incomplete extraction of hemicellulose with water, it is not possible to assess the extent of the association of acetyl group with any fibre component, particularly alpha-cellulose, other than hemicellulose. Although the major portion of acetyl group in jute fibre appears to be in combination with the carbohydrate fraction, the possibility of its association with hemicellulose is yet to be proved by direct experimental evidence. It was, therefore, considered of importance to devise a suitable method for isolating jute hemicellulose with its acetyl content intact, as far as possible, and also to ascertain whether the acetyl group is associated only with different components of hemicellulose or to some extent with alpha-cellulose.

Experimental procedure

Preparation of materials — A sample of good quality white jute obtained from Pakistan was used in the investigation, the fibre being collected from the middle portions of the strands after rejecting 1·5 ft lengths from both ends. The material was cut to small pieces (0·5 in. lengths), dewaxed by extracting with alcohol-benzene mixture (1: 2), washed with alcohol and water, and then air-dried.

Holocellulose was isolated from dewaxed jute by the action of sodium chlorite solution (0·7 per cent) adjusted to *p*H 4 with acetic acid-acetate buffer, using a liquor ratio of 50:1, for 2 hr at 98-99°C., followed by washing with sodium bisulphite solution (2 per cent) and finally dried in the room atmosphere.

Extraction of crude hemicellulose — The hemicellulose was extracted with water from the isolated holocellulose by two different methods, namely under reflux and under pressure.

In the former procedure, the sample of holocellulose in two lots, each of 50 g., was subjected to five successive extractions with boiling water (1250 ml. for each extraction) under reflux for periods of 2, 4, 6, 8 and 8 hr respectively. The latter method of extraction was carried out by digesting duplicate samples of holocellulose (50 g.) with water in an autoclave under a pressure of 20 lb./sq. in., the material being subjected to five successive extractions, each of 4 hr duration. The individual extracts obtained by both the methods were separately adjusted to *p*H 7 with dilute sodium hydroxide solution, evaporated to near dryness under reduced pressure and finally dried over phosphorus pentoxide *in vacuo* before weighing.

Purification of crude hemicellulose — The extracts obtained by each of the above methods were mixed together so as to obtain two representative samples of crude hemicellulose. Purification of the crude product was effected by dissolving a portion of each of the samples (10 g.) in a minimum amount of water and reprecipitating by the addition of alcohol. The sample obtained by refluxing was reprecipitated by adding 5-volume alcohol since only a slight turbidity was produced at a lower concentration of alcohol, whereas two crops were isolated from the other sample, the first in the presence of 3-volume and the second with 5-volume alcohol. The precipitated hemicellulose was collected by centrifugation, washed with alcohol followed by ether, and finally dried over phosphorus pentoxide *in vacuo*.

Chemical analysis of hemicellulose — The carbon dioxide due to uronic acid was determined from its rate of evolution following the method employed by Macmillan and Sen Gupta[3] and furfural on distilling with 13·13 per cent hydrochloric acid by a modified procedure of Kullgren and Tydén[9]. Acetyl was determined from the amount of acetic acid evolved on steam distilling the pre-hydrolysed acidified product[3], and methoxyl by the volumetric semi-micro method described by Dorée[10].

The hemicellulose contents of holocellulose before and after five extractions with water under pressure, as described above, were determined from the loss in weight suffered by treatment with 9·5 per cent sodium hydroxide under the conditions of estimating alpha-cellulose[11].

Paper chromatographic analysis of hemicellulose — The sample (0·2 g.) was hydrolysed by refluxing with *N* sulphuric acid for 6 hr, the solution neutralized with barium carbonate, and the mixture centrifuged, the clear liquid obtained being concentrated to a small bulk at 40°C. The hydrolysate was then chromatographed on Whatman No. 1 filter paper with acetic acid-*n*-butanol-water as the solvent mixture, following the technique employed by Macmillan *et al.*[12]; the sugars were detected with aniline hydrogen phthalate reagent.

Results and discussion

The yield of cation-free holocellulose obtained from the sample of jute under investigation was 88·28 per cent. The extraction of hemicellulose with water from holocellulose was more thoroughly and easily effected under pressure than under reflux. It was found that two successive extractions for a total period of 6 hr under the latter set of conditions resulted in the isolation of 6·3 per cent crude hemicellulose, whereas under the former set of conditions, only a single treatment for 4 hr gave a yield as high as 16·5 per cent. Approximately 90 per cent of the hemicellulose was extracted by five successive treatments under pressure for a period of 20 hr but the same number of treatments under reflux for 28 hr effected extraction to the extent of about 68 per cent.

The samples of crude hemicellulose obtained both by treatment under reflux and pressure were dark brown solids; the purified products were amorphous-white substances with a slight brownish tinge. The crude products were found to be contaminated with appreciable amounts of lignin, as was evident from the characteristic colour test (Mäule), the purified materials being almost free from ligneous impurities.

The analytical data along with the yields of the crude and the purified fractions of hemicellulose are given in Table 1.

The results obtained show that there is a general tendency for the uronic-carbon dioxide of the crude hemicellulose to increase and the methoxyl content to decrease on purification, indicating thereby that substances associated with certain amounts of methoxyl, possibly lignin and its degradation products, have been eliminated during purification.

Since acetyl group occurs in each and every fraction of the water-extracted hemicellulose, it appears that this is a characteristic functional group constituting an integral part of jute hemicellulose. This observation has so long been overlooked, because the extraction of hemicellulose in the past was confined to the treatment with alkali only, which readily hydrolysed and eliminated this group as sodium acetate.

It has already been demonstrated by Sarkar *et al.*[13] that uronic acid in jute hemicellulose occurs mainly as the monomethyl derivative which is incapable of yielding any furfural on distillation with acid. In

TABLE 1 — YIELDS AND ANALYSES OF WATER-EXTRACTED HEMICELLULOSE

Fraction No.	Method of preparation	Yield %	Uronic-CO_2 %	Furfural %	Acetyl %	Methoxyl %
1	Crude product by refluxing	17·05	4·26	37·57	7·00	5·68
1a	Purified product by precipitation with 5-vol. alcohol from fraction 1	12·42	4·75	34·33	7·45	3·62
2	Crude product by pressure extraction	22·79	3·69	37·63	8·10	5·40
2a	Purified product by precipitation with 3-vol. alcohol from fraction 2	12·61	4·62	35·55	8·76	3·66
2b	Purified product by precipitation with 5-vol. alcohol from filtrate of fraction 2a	5·10	2·94	36·61	9·55	4·32

Yield expressed on oven-dry holocellulose and other results on respective hemicellulose fractions dried over P_2O_5.

addition, since only traces of other pentose residues are likely to be present in jute hemicellulose, the yield of furfural obtained may be assumed to be derived, for all practical purposes, entirely from xylan. The results of the present investigation, therefore, are in general agreement with those reported previously[13] that jute hemicellulose may be considered to be composed mainly of chains of monomethyl uronic acid and xylan, although small amounts of non-methylated uronic acid residue and hexosan may also occur. The water-extracted hemicellulose, however, was found to contain a smaller amount of furfural-yielding substance, i.e. xylan, than that reported earlier[13] for the sample isolated from an alkaline extract of holocellulose.

The possibility of the existence of non-methylated uronic acid and hexosan in the water-extracted hemicellulose is confirmed by the paper chromatographic examination (Table 2).

From the dimensions and colour intensities of spots for different sugars identified on the chromatogram, it appears that xylose constitutes the major portion of each of the fractions, whereas hexose residues in different fractions, comprising mainly galactose with occasional presence of glucose and traces of mannose, occur in relatively small proportions. In addition, all the fractions have been found to contain certain amounts of rhamnose and non-methylated uronic acid which will invariably yield furfural on distillation with acid. The fraction of uronic acid, which on hydrolysis liberates the free non-methylated acid, appears to involve a different mode of linkage from that of the major fraction with component sugar residues because hydrolysis of the compound with the latter type of linkage yields, instead of free uronic acid, an aldobiuronic acid[13] undetectable by the paper chromatographic examination.

The acetyl contents of the unextracted holocellulose and the residual material left after five extractions with water under pressure have been found to be

TABLE 2 — SUGAR COMPONENTS OF PURIFIED FRACTION OF HEMICELLULOSE

Sugar component	Fraction No.		
	1a	2a	2b
Barium uronate	+	+	+
Galactose	+	—	+
Glucose	+	+	+
Mannose	Traces	Traces	—
Xylose	+	+	+
Rhamnose	+	+	+

+ indicates the presence and —, the absence of sugar component.

3·45 per cent and 0·58 per cent (on unextracted sample) respectively, while analysis of the extracted crude hemicellulose accounts for only 1·85 per cent acetyl. It is obvious, therefore, that a considerable portion of the group (1·02 per cent) has been hydrolysed and subsequently removed along with steam during the process of extracting hemicellulose. The maximum possible amount of acetyl which could exist in combination with the isolated hemicellulose (90 per cent) would have amounted to 2·87 per cent, had the entire water-extractable acetyl been associated with the hemicellulosic component and had there been no loss of the acetyl group during the process of extraction. On the assumption that acetyl group is uniformly distributed in the hemicellulosic fraction, the portion (10 per cent) present in the water-extracted residual holocellulose is expected to contain 0·32 per cent acetyl group, and retention of a greater amount (0·58 per cent) suggests the possibility that a small portion of the group is in association with alpha-cellulose.

Although the acetyl group appears to occur mostly in the hemicellulosic fraction, owing to complexity of the chemical composition of the latter, it is rather difficult to visualize with which sugar residues the acetyl group is actually associated. Since it has been shown[14] that hexosans are present in jute

holocellulose to a maximum extent of 1·10 per cent, a simple calculation reveals that only a small fraction of acetyl group (0·88 per cent) can be accounted for, even if it is assumed that three groups of acetyl occur per hexose residue of the chain. It appears probable, therefore, that the major portion of the acetyl group in combination with hemicellulose is not associated with hexosans but with either xylose or uronic acid residue or both.

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Chemical Examination of *Centella asiatica* Linn.: Part I— Isolation of the Chemical Constituents

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The chemical examination of *Centella asiatica* Linn. has resulted in the isolation of six constituents in pure state: two saponins, brahmoside and brahminoside; two triterpene acids, brahmic acid and isobrahmic acid; betulic acid and stigmasterol. The chemical nature of brahmic acid has been established and it has been shown that brahmic acid contains one carboxyl, 3 acetylable hydroxyls and one double bond, and belongs to α-amyrin group. Brahmic acid has been found to be different from indocentoic acid isolated by earlier workers from the Indian variety of the same plant.

CENTELLA asiatica Linn. (syn.: *Hydrocotyle asiatica* Linn.; N.O.: *Umbelliferae*) is reputed to be useful in the treatment of leucoderma, anaemia, inflammations, smallpox and asthma. It is regarded to be a sedative, cardiotonic and is used in the indigenous systems of medicine for improving memory and in certain forms of insanity[1].

The Madagascar variety of this plant has been investigated by Polonsky and co-workers[2,3]. They isolated a crystalline glycoside, asiaticoside, m.p. 230-3° (decomp.), which was shown to possess some activity against human leprosy[4]. On hydrolysis, asiaticoside gave asiatic acid, $C_{30}H_{48}O_5$, m.p. 240-4°, glucose and rhamnose. These authors showed that asiatic acid was 2,3,23-trihydroxy-28-carboxy-α-amyrene[5]. Recently, a complete structure for asiaticoside has been proposed[6].

Bhattacharyya and Lythgoe[7] examined the *C. asiatica* varieties found in Ceylon and India. From the Ceylonese variety[8] they isolated three triterpenic acids, centic acid, $C_{30}H_{48}O_5$, m.p. 230-55° (decomp.); centoic acid, $C_{30}H_{48}O_6$, m.p. 245-61° (decomp.) and centellic acid, $C_{30}H_{48}O_6$, m.p. 160-95° (decomp.). From the Indian variety, Bhattacharyya[9] has

reported the isolation of indocentoic acid, $C_{30}H_{48}O_6$, m.p. 272°. Preliminary examination has also been carried out on the Indian variety by other workers. Wali and Tumminkatti[10] have reported the presence of sitosterol (m.p. 137°) and a resinous substance, and Basu and Singh[11] have isolated an alkaloid, hydrocotylin, in 0·0016 per cent yield.

In view of the importance of this plant in the indigenous system of medicine, it was considered desirable to take it up for a detailed study. Work so far has, in our hands, resulted in the isolation of the following products in a pure state:

	M.P. °C.	Yield %
Brahmoside	242 (decomp.)	0·370
Brahminoside	223 (decomp.)	0·160
Brahmic acid	293	0·097
Isobrahmic acid	263	0·090
Betulic acid	308	0·110
Stigmasterol	170	0·004

The alcoholic concentrate from the plant material was extracted with benzene. The benzene extractive deposited a precipitate which was separated. The benzene-insoluble alcoholic concentrate was partitioned between benzene and 60 per cent alcohol, which showed subsequently to have established a good separation of the triterpene acids in the benzene layer and of the saponins in the alcohol layer. The total benzene solution gave a powder on concentration and dilution with petrol ether.

Attempts were made to purify these two powders separately by fractional precipitation from alcohol. This procedure resulted in a number of fractions melting with decomposition within a range of 240-70°. Similar results were obtained by chromatography over silica gel. Attempts at purification by chromatography over IR-400 and IR-45 amberlite resins, and via the brucine salt led to a number of fractions and subfractions which melted between 253° and 280° (decomp.). These purification procedures did not, however, lead to any pure products. Finally, all the fractions were mixed together and worked up by the following method. The alkali-insoluble material was separated by treatment with dilute caustic potash. The free acids were liberated from the alkaline filtrate by acidification, and their chromatography over alumina gave two distinct fractions, one eluting with alcohol and the other with alcoholic acetic acid (2 per cent). The alcohol eluate gave on dilution with acetone a colourless powder, m.p. 293°, which has been provisionally named as brahmic acid. The mother liquor contained some betulic acid as was indicated by its infrared absorption spectrum. The major portion of betulic acid was, however, present in the benzene-petrol ether solution. The alcohol-acetic acid eluate gave, on purification with dilute alcohol, a colourless powder, m.p. 263°, and this has been designated as isobrahmic acid.

The residue from the total benzene-petrol ether solution was saponified and the unsaponifiable matter chromatographed over alumina in benzene solution and 59 fractions were collected as detailed under the section 'Experimental procedure'. The benzene eluates (2 and 3) gave, on repeated fractional crystallization from alcohol, a crystalline sterol, m.p. 164°. This was found to be a mixture of stigmasterol and sitosterols. The former was separated from the mixture as ether-insoluble tetrabromide and identified on the basis of its m.p., 170°, the m.p. of its acetyl derivative, 143°, and their optical rotations.

The ethyl acetate-alcohol eluates (40-48) gave a colourless powder from alcohol, m.p. 308°. This was identified as betulic acid on the basis of its physical and chemical properties.

The 60 per cent alcoholic fraction, obtained earlier, was concentrated to dryness. The pasty mass was freed of the water-soluble material by trituration with cold water. The water-insoluble portion was taken into alcohol and precipitated with ether and petrol ether. This led to the separation of a water-soluble dark brown residue which was mixed with the aqueous extract. Both the alcohol ether-petrol ether solution and the aqueous layer were purified separately by the conventional lead acetate and lead carbonate treatment followed by the removal of the inorganic material by partitioning the ultimate residue between butanol and water. This gave pale coloured powders of crude saponins (87 and 20 g. respectively).

The major fraction (87 g.) was chromatographed over silica gel. On the basis of melting points and the R_f values of each eluate on paper chromatogram[12], a fraction was obtained which melted at 218-22° (decomp.) and showed two spots on paper chromatogram (R_f 0·23 and 0·67). This powder was then subjected to charcoal chromatography when the separation of two components was effected. Eluates 4 and 5 were still heterogeneous and gave the same two spots, but the powders from eluates 6 to 20 showed only one spot, R_f 0·67, and melted at 241-2° (decomp.). These were, therefore, mixed and the pure compound provisionally named as brahmoside.

The minor fraction (20 g.) showed only one spot on paper chromatography, R_f 0·23, and was purified by dissolving it in alcohol and fractional precipitation with acetone. It melted finally at 223° (decomp.) and has been named as brahminoside.

Brahmic acid and isobrahmic acid were found to retain water very tenaciously and the samples were, therefore, dried progressively at higher temperatures

till concordant values for carbon and hydrogen analysis were obtained. Both the acids analysed for $C_{30}H_{48}O_6$.

Brahmic acid gave the characteristic Noller's[13] colour reaction but did not give yellow colour with tetranitromethane. It formed a triacetyl derivative and gave a crystalline methyl ester after chromatography over alumina. It was found that the ester may be obtained under identical experimental conditions with a lower melting point. As is shown in the section 'Experimental procedure', this behaviour is due to the retention of water. The methyl ester is resistant to alkaline hydrolysis and forms a triacetyl derivative. The acid gave a positive iodoform reaction indicating a primary hydroxyl group and this was confirmed by the formation of a monotrityl derivative[14]. Thus five oxygen functions are accounted for: two in the carboxyl function, one primary hydroxyl and two secondary hydroxyls. The sixth oxygen may be present either as a tertiary hydroxyl or a hindered secondary hydroxyl function. The acid consumes 1 mole of periodate indicating the presence of a glycol moiety in the molecule.

The presence of a trisubstituted double bond in brahmic acid was shown by bands at 3030 cm.$^{-1}$ and 1660 cm.$^{-1}$ in the infrared spectrum and proved conclusively by chromic acid oxidation of methyl brahmate triacetate; the resultant compound showed maxima at 249 mμ characteristic for αβ-unsaturated ketones. In analogy with the known acids, the position of the double bond is, therefore, fixed at carbon 12-13. Since methyl brahmate does not consume perbenzoic acid and the triacetate does not show characteristic triple ultraviolet absorption maxima at 240·5, 249 and 258 mμ due to a $\Delta^{11,13(18)}$ diene after oxidation with selenium dioxide[15], brahmic acid belongs to the α-amyrin group.

Brahmic acid is different from the indocentoic acid isolated by Bhattacharyya. The former melts at 293° and the latter at 272°. It may be pointed out that indocentoic acid forms only a diacetyl derivative whereas brahmic acid gives a triacetate. In the present investigation we were unable to obtain indocentoic acid.

Further work on the constitution of the different products is in progress.

Experimental procedure

The air-dried plant powder (10 kg.) was exhaustively extracted with alcohol and the total extract concentrated at 50° under reduced pressure. The concentrate deposited, on cooling, inorganic material which was filtered off. The filtrate was concentrated and extracted with benzene. The benzene solution was kept in the cold when a precipitate (I) (13·4 g.), m.p. 269-70° (decomp.), was obtained. The benzene-insoluble residue was partitioned between 60 per cent alcohol (II) and benzene. The latter was added to the earlier benzene extract and the total benzene solution concentrated to about 1 litre, and diluted with the same volume of petrol ether, when a gelatinous precipitate was obtained and separated by centrifugation. The precipitate was charcoaled in alcoholic solution, concentrated and precipitated with a large excess of acetone, when a powder (25·8 g.), m.p. 245-67° (decomp.) (III), was obtained. The mother liquor was added to the benzene solution (IV).

The powders (I) and (III) were dissolved separately in alcohol and charcoaled to remove chlorophyll and other coloured impurities. The alcoholic filtrate was in each case fractionally precipitated to give six fractions. Each fraction, however, melted in a range of 240-70°. Similar indefinite melting fractions were obtained after silica gel chromatography of the powder in ethyl acetate-methanol (9:1) and elution with ethyl acetate containing increasing quantities of methanol.

Finally, all the fractions from both the powders were combined and macerated with 5 per cent aqueous potash, filtered and the insoluble residue (1·7 g.), m.p. 273-8°, washed with water. The total filtrate was acidified and the precipitated acids filtered (24 g.). The precipitate was dissolved in alcohol-tetrahydrofuran (THF) (2:1) and chromatographed over alumina (500 g.). The yields and melting points of the products isolated from various eluates are given in Table 1.

Eluates 2, 3 and 4 (Table 1) gave colourless powders (13·8 g.), which were mixed together, dissolved in alcohol and fractionally precipitated with acetone. This resulted in three fractions each melting at 289-91°. Final crystallization from alcohol gave pure brahmic acid (9·7 g.), m.p. 293°. A further quantity of the powder (2·6 g.), m.p. 265-75°, was obtained from the mother liquor. Its infrared absorption spectrum showed a characteristic band for the methylene group at 892 cm.$^{-1}$.

The total powder from eluates 6 to 12 (10·3 g.) was dissolved in alcohol and precipitated fractionally with water. The three different fractions so obtained melted at 262°, 259° and 258° respectively. On subsequent crystallization of the combined fractions from dilute alcohol, isobrahmic acid (9 g.), m.p. 263°, was obtained.

The total benzene-petrol ether solution (IV) was freed of the solvent and saponified with 10 per cent alcoholic potash. After usual working, the mixture was extracted with ethyl acetate and the residue from the ethyl acetate layer (43·6 g.) chromato-

TABLE 1 — CHROMATOGRAPHY OF THE FREE ACIDS

ELUATE No.	ELUANT	YIELD g.	M.P.* °C.
1	Ethanol-THF (2: 1)	0·70	255-6
2	Ethanol-THF (1: 1)	1·02	278
3	Ethanol	11·94	278
4	Ethanol	0·92	276
5	Ethanol	1·12	265-9
6	Ethanol-acetic acid (2%)	0·78	256-65
7-12	Ethanol-acetic acid (2%)	10·34	256-65

*Fractions melted with decomposition.

TABLE 2 — CHROMATOGRAPHY OF UNSAPONIFIABLE MATTER FROM TOTAL BENZENE-PETROL ETHER SOLUTION

ELUATE No.	ELUANT	YIELD g.	APPEARANCE
1	Benzene	6·52	Pale oil
2	Benzene	5·20	Viscous oil with crystals
3	Benzene	2·66	Crystalline
4	Benzene	0·48	Resinous
5	Benzene	0·30	do
6-8	Benzene-ether (9: 1)	0·76	do
9-11	Benzene-ether (4: 1)	0·42	do
12-14	Benzene-ether (2: 1)	0·22	do
15-17	Benzene-ether (1: 1)	0·26	do
18-20	Ether	0·04	do
21-26	Ether-methanol (9: 1)	1·50	do
27-32	Ether-methanol (4: 1)	0·14	do
33-37	Ethyl acetate-ethanol (9: 1)	0·32	do
38-39	Ethyl acetate-ethanol (4: 1)	0·14	do
40-49	Ethyl acetate-ethanol (1: 1)	13·99	Powder
50-51	Ethanol-acetic acid (1%)	0·46	Resinous
52-55	Ethanol-acetic acid (1%)	14·60	Solid
56-59	Ethanol-acetic acid (2%)	1·62	Resinous

graphed over alumina (2 kg.). Fifty-nine fractions were collected (Table 2).

Eluates 2 and 3 (Table 2) gave, on repeated fractional crystallization from alcohol, colourless shining plates (1·2 g.), m.p. 164° (fraction 164).

Eluates 40-48 gave powders melting in the range of 270-92° (decomp.). These were mixed and crystallized fractionally from alcohol, when a colourless powder was obtained (11·6 g.), m.p. 308-9° (compound 308).

The 60 per cent alcoholic extractive (II) was freed of the solvent under reduced pressure. The residue (310 g.) was macerated in ice-cold water, the insoluble portion dissolved in alcohol and precipitated with ether and petrol ether. Repetition of this procedure gave a dark brown alcohol-insoluble resinous mass. This and the aqueous extract, obtained earlier, were mixed together (V).

The alcohol-ether-petrol ether solution was freed of the solvent, dissolved in 40 per cent alcohol (3 litres) and treated with a 10 per cent aqueous lead acetate and lead carbonate solution. The precipitated lead salts were filtered and the filtrate freed of the lead salts by treatment with hydrogen sulphide. The *p*H of the filtrate was adjusted to 6·6 with potassium carbonate solution and the solution concentrated under reduced pressure. Inorganic materials were removed from the residue by dissolving it in butanol and extracting repeatedly with water. The butanol solution was concentrated in vacuum to a small volume and the total saponins (colourless powder, 87 g.) precipitated by adding excess of acetone.

The crude saponin powder was chromatographed over silica gel (2 kg.) in ethyl acetate-alcohol (40:60) solution. The fractions obtained, the melting points and yields of products are given in Table 3. The powders, obtained from eluates 1 to 5, melted at 218-22° (75·57 g.) and each showed two spots (R_f 0·23 and 0·67) on paper chromatography using butanol-ethyl acetate-water (4: 1: 5) solvent system and 25 per cent solution of trichloroacetic acid in ether as the developer. These were, therefore, mixed and rechromatographed on a charcoal column (750 g.) in alcohol-water (1: 1) and the fractions collected are listed in Table 4.

The powders obtained from each eluate were simultaneously chromatographed on paper. Eluates 1 to 3 did not show any spot. Eluates 4 and 5 showed two spots, R_f 0·23 and 0·66, while those from 6 to 21 showed only one spot, R_f 0·66. Eluates 22-24 also gave only one spot, R_f 0·67. Powders from

TABLE 3 — CHROMATOGRAPHY OF CRUDE SAPONINS

ELUATE No.	ELUANT	YIELD g.	M.P.* °C.
1-4	Ethanol-ethyl acetate (60: 40)	71·81	218-22
5	Ethanol-ethyl acetate (70: 30)	3·76	218-22
6	Ethanol-ethyl acetate (70: 30)	0·50	225-31
7-9	Ethanol-ethyl acetate (80: 20)	0·94	230
10-11	Ethanol	0·50	230
12-13	Ethanol-water (1: 1)	0·89	—
14	Water	2·81	—

*Fractions melted with decomposition.

TABLE 4 — CHROMATOGRAPHY OF FRACTIONS 1-5* OVER CHARCOAL

ELUATE No.	ELUANT	YIELD g.
1-3	Ethanol-water (1: 1)	0·47
4-5	Ethanol-water (2: 1)	22·00
6-7	Ethanol-water (2: 1)	25·50
8-10	Ethanol-water (3: 1)	9·06
11-13	Ethanol-water (5: 1)	4·75
14-16	Ethanol-water (8: 1)	3·62
17-21	Ethanol	2·69
22-24	Ethanol-pyridine (1: 1)	1·26

*Fractions 1-5 refer to those obtained by chromatography of crude saponins over silica gel and listed in Table 3.

eluates 6 to 21 (m.p. 241-2° decomp.) were mixed, dissolved in absolute alcohol, filtered and precipitated with ether. The pure saponin (brahmoside) was thus obtained as colourless powder (37·5 g.), m.p. 242° (decomp.).

The total aqueous solution (V), obtained above, was extracted repeatedly with butanol. The butanol layer was freed of the solvent under reduced pressure and the residue treated with lead acetate and lead carbonate in aqueous solution. After working in the usual manner, the butanol solution was concentrated and diluted with excess of acetone, when a yellowish powder (20 g.), m.p. 211-2° (decomp.), was obtained. It showed only one spot on paper chromatography, R_f 0·23. It was dissolved in alcohol and again fractionally precipitated with acetone, when a colourless powder of brahminoside (16 g.), m.p. 223° (decomp.), was obtained.

Brahmic acid — Colourless powder, m.p. 293°, fairly soluble in alcohol and methanol but insoluble in the rest of the solvents. It gives a red colour with thionyl chloride which deepens gradually to deep violet-red. In Liebermann-Burchard reaction, it gives a light brown colour which develops violet shade after 5-10 min. It gives no colouration with tetranitromethane. It is soluble in dilute alkali and insoluble in acids; $[\alpha]_D^{20}$, +24° (pyridine). The analytical sample was dried at 133° and finally at 155° to get concordant analytical results. (Found: C, 71·14; H, 10·0%; eq. wt, 509. $C_{30}H_{48}O_6$ requires C, 71·4; H, 9·5%; mol. wt, 504.)

Brahmic acid triacetate — Brahmic acid (200 mg.) was dissolved in acetic anhydride (1 ml.) and dry pyridine (0·5 ml.) and kept at room temperature for 48 hr. The mixture gave, on working as usual, a powder which was chromatographed on silica gel in petrol ether-benzene (5: 1) solution. The ethyl acetate eluate yielded a colourless powder (175 mg.), m.p. 172° (decomp.), $[\alpha]_D^{20}$, +19·5° (chloroform). [Found: C, 68·8; H, 8·9; acetyl, 20·2. $C_{36}H_{54}O_9$ requires C, 68·5; H, 8·6; acetyl (3), 20·4%.]

Methyl brahmate — Brahmic acid (500 mg.) was suspended in ether (40 ml.) and diazomethane passed at 0°. After 48 hr at room temperature, the reaction solution was filtered and freed of the solvent. The residue was chromatographed over alumina in benzene solution, and eluted successively with benzene, ether, ether-ethyl acetate (1: 1), ethyl acetate, ethyl acetate-methanol (7: 3) and finally methanol.

The residue obtained by eluting with ethyl acetate-methanol (7: 3) was in the form of colourless needles, m.p. 213°, $[\alpha]_D^{20}$, +34° (chloroform).

The samples dried over xylene gave: C, 71·77; H, 10·0. $C_{31}H_{50}O_6$ requires C, 71·8; H, 9·6%. A lower melting product was obtained from the successive working. This was found to retain water which could, however, be removed by drying at 133° in vacuum. Methyl brahmate (30 mg.) was recovered unchanged after treatment with 8 per cent alcoholic alkali for 1·5 hr. It did not show any uptake of perbenzoic acid after keeping for 70 hr at 0° in chloroform solution.

Methyl brahmate triacetate — Methyl brahmate (100 mg.) was acetylated with acetic anhydride and pyridine as usual and the acetyl derivative dissolved in alcohol and precipitated with water giving a colourless powder (110 mg.), m.p. 127-30° (decomp.). (Found: C, 68·65; H, 9·37; acetyl, 18·86. $C_{37}H_{56}O_9$ requires C, 68·98; H, 8·8; acetyl (3), 20·0%.)

Attempted oxidation of the triacetate with selenium dioxide, in glacial acetic acid, gave back the original compound, m.p. 125-30° (decomp.), which did not depress the melting point of the original substance.

Methyl brahmate triacetate (70 mg.) was dissolved in glacial acetic acid and heated at 110°. Chromic acid (100 mg.) was then added gradually in 30 min. and heating continued for 1 hr. The reaction mixture was diluted with water, the precipitated derivative filtered, and chromatographed over silica gel in benzene solution. The compound eluted out with ether (50 mg.), m.p. 206-8°. It showed $\lambda_{max.}$, 249 mμ; log ϵ = 3·76. (Found: C, 67·0; H, 8·26. $C_{37}H_{56}O_{10}$ requires C, 67·2; H, 8·5%.)

Periodate oxidation of methyl brahmate — Methyl ester (33·8 mg.) was dissolved in ethyl acetate-alcohol (1: 1) mixture (2 ml.) and sodium periodate solution (1·5 ml., 32·7 mg.) added to it. After 10 hr the excess of periodate was determined with 0·1*N* sodium arsenite in the usual manner and found to be 20·8 mg.

Sodium periodate consumed, 12·9 mg.; required for 1 mole, 13·6 mg.

Monotrityl methyl brahmate — Methyl brahmate (100 mg.) and trityl chloride (300 mg.) in dioxane-pyridine (1: 1) mixture were heated on water bath for 8 hr and left overnight. The mixture was diluted with water and resultant precipitate filtered. The precipitate was dissolved in benzene and chromatographed over alumina. The trityl derivative eluted out with chloroform-ethyl acetate (3: 1), m.p. 189-91°. (Found: C, 79·03; H, 8·66. $C_{50}H_{64}O_6$ requires C, 79·0; H, 8·42%.)

Fraction 164 — This fraction (1·2 g.) was acetylated with acetic anhydride and pyridine. The ethereal solution of the acetyl derivative (m.p. 137°) was treated with bromine in glacial acetic acid for 12 hr when a crystalline bromide (m.p. 203°) separated out and was filtered.

The bromide (0·88 g.) was refluxed with zinc dust in glacial acetic acid for 2 hr. A precipitate was

obtained by dilution of the mixture with water and was crystallized from alcohol. The acetyl derivative (450 mg.) melted at 143°, $[\alpha]_D^{20}$, −55° (chloroform). (Found: C, 81·68; H, 11·25. $C_{31}H_{50}O_2$ requires C, 81·9; H, 11·0%.)

The acetyl derivative was refluxed with 10 per cent alcoholic potash for 2 hr. The mixture was diluted with water and the precipitate filtered. It crystallized from alcohol in plates (380 mg.), m.p. 170°. It gives with acetic anhydride and sulphuric acid a deep blue colour which turns green. In Salkowski reaction the sulphuric acid becomes deep orange while chloroform layer remains colourless, $[\alpha]_D^{20}$, −51° (chloroform). (Found: C, 84·23; H, 11·91. $C_{29}H_{48}O$ requires C, 84·4; H, 11·6%.)

The mother liquor from bromination reaction, obtained above, gave a powder which was similarly treated with zinc dust in glacial acetic acid. The resultant acetyl derivative (m.p. 132°) was deacetylated with alcoholic potash and the product obtained in colourless needles (520 mg.), m.p. 139-45°. In Liebermann-Burchard reaction it showed colour changes from red → violet → blue → green. In Salkowski reaction the sulphuric acid layer turns orange.

Compound 308 — Colourless powder melting at 308-9°. It is soluble in ethyl acetate, alcohol and methanol, fairly so in acetone and chloroform. It gives a red colour with thionyl chloride which deepens to violet. In Liebermann-Burchard reaction, it gives a deep violet colouration, $[\alpha]_D^{20}$, +4·0° (pyridine). (Found: C, 78·69; H, 10·55. $C_{30}H_{48}O_3$ requires C, 78·9; H, 10·54%.)

Acetyl derivative — Compound 308 (200 mg.) was acetylated with acetic anhydride and pyridine and worked up in the usual manner. The powder crystallized from alcohol as colourless needles (180 mg.), m.p. 294°, $[\alpha]_D^{20}$, +12·0° (pyridine). (Found: C, 77·19; H, 10·28. $C_{32}H_{50}O_4$ requires C, 77·0; H, 10·0%.)

Methyl ester of compound 308 — Compound 308 (400 mg.) was suspended in ether and treated with excess of ethereal diazomethane. After 48 hr, ether was evaporated off and the residue crystallized from alcohol, colourless needles (300 mg.), m.p. 222°, $[\alpha]_D^{20}$, +11·0° (pyridine). (Found: C, 78·87; H, 10·45. $C_{31}H_{50}O_3$ requires C, 79·1; H, 10·65%.)

Acetyl derivative of methyl ester of compound 308 — The methyl ester (100 mg.) was acetylated in the usual manner and the powder crystallized from alcohol, colourless needles (80 mg.), m.p. 202°. (Found: C, 77·5; H, 10·38. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·1%.)

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A Study of Transformations in the Coumaran-3-one Derivatives

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A new synthesis of 2-benzylcoumaranones is described involving benzylation of 2-acetylcoumaranones and subsequent deacetylation. N-Bromosuccinimide has been found to be the most satisfactory for the dehydrogenation of 2-benzyl-coumaranones to aurones.

Demethylation of *o*-hydroxy-ω-methoxyacetophenones with hydrobromic acid at 100° gives rise to the corresponding ω-hydroxy compounds and not coumaran-3-ones. The condensation products of the latter with benzaldehyde are 2-hydroxy-2-benzylcoumaranones.

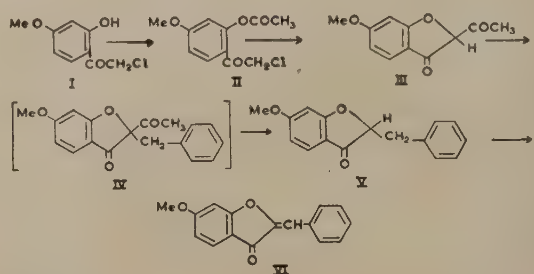
Partial methylation of 4,6-dihydroxycoumaran-3-one is most satisfactorily done in dioxan-benzene medium and occurs in the 4-position. This is supported by a new synthesis of the 4-methyl ether. The same solvent is good for complete methylation also; acetone is unsuitable as it forms a condensation product involving the 2-methylene group yielding *bis*-dimethoxycoumaranonyl dimethyl methane.

Methylation and ultraviolet studies of 4,6-dihydroxycoumaran-3-one and aurone indicate that there is no hydrogen bonding between 4-hydroxyl and 3-carbonyl groups. Partial selective methylation is not possible in the case of 4,6-dihydroxyaurone.

COUMARANONES and their derivatives are becoming more and more important in the study of the chemistry of plant pigments. The preparation of compounds of this group and the study of their behaviour during methylation are discussed in this paper.

Preparation of 2-benzylcoumaranones and dehydrogenation to aurones

The preparation of 2-benzylcoumaranones (V) by the hydrogenation of the corresponding benzylidene coumaran-3-ones (VI) in the presence of metallic catalysts has been reported earlier[1-3]; it involves the preparation of an aurone by the action of benzaldehyde on the appropriate coumaran-3-one[4,5]. An alternative method is through the Friedel-Crafts reaction using α-bromo-β-phenylpropionyl chloride and the appropriate phenol methyl ether[6]. The preparation would be simpler if benzylation of coumaran-3-one itself could be carried out. But this does not take place[6] though the 2-methylene group is active enough to condense with aldehydes and ketones. It was, therefore, considered necessary to activate the methylene group by introducing an acetyl group (III). This cannot be achieved by direct acetylation, but it is easy to synthesize such compounds by the methods reported earlier by Auwers[7] and Jones *et al.*[8]. As a typical example, 2-acetyl-6-methoxy-coumaranone (III) has now been prepared by the acetylation of ω-chloropeonol (I) with acetic anhydride and perchloric acid and subsequent Auwers migration accompanied by cyclization. The migration of the acetyl group takes place with ease because the concerned methylene group is activated by the ω-chlorine atom. The mechanism of such migrations is analogous to that of Baker-Venkataraman transformation[8,9]. This acetyl derivative undergoes smooth



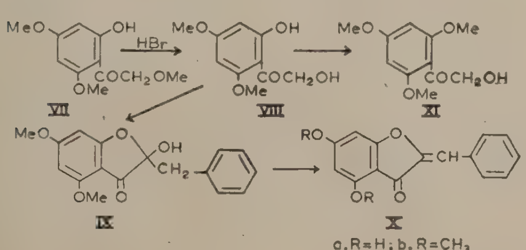
benzylation with benzyl chloride and sodium ethoxide. Though the initial product (IV) could not be isolated in the crystalline state, it gave rise to 2-benzyl-6-methoxy coumaranone (V) by deacetylation with boiling aqueous sodium carbonate.

Since the 2-benzylcoumaranone was thus conveniently obtained, dehydrogenation to aurone was next examined. Earlier this was effected by the oxidation of 2-benzylcoumaranone with neutral potassium permanganate in acetone medium to 2-hydroxy-2-benzylcoumaranone and subsequent dehydration with concentrated sulphuric acid[3]; this method gives rather poor yield (10 per cent). To improve the yields, N-bromosuccinimide has now been employed and it gives 6-methoxyaurone (VI) in 80 per cent yield.

Demethylation of ω-methoxy-*o*-hydroxyacetophenones

Balakrishna *et al.*[10] reported that by the action of hydrobromic acid on ω-methoxy-*o*-hydroxyacetophenones, coumaran-3-ones were formed. In studying the suitability of this method, the action of this reagent on 2-hydroxy-ω-4,6-trimethoxyacetophenone (VII) was re-examined. The product melts at 140° which is close to the temperature 147-8° recorded earlier[10]. But it does not agree with the properties of a coumaran-3-one, though it also reacts with benzaldehyde in the presence of alkali. The carbon, hydrogen and methoxyl analyses agree with the molecular formula of ω-2-dihydroxy-4,6-dimethoxyacetophenone (VIII). The product could be methylated with dimethyl sulphate and potassium carbonate in acetone medium to yield ω-hydroxy-2,4,6-trimethoxyacetophenone (XI). The condensation with benzaldehyde takes place in alkaline medium to yield a compound of molecular formula $C_{17}H_{16}O_5$, m.p. 171-2°, which agrees with *apo*alpinone methyl ether (2-hydroxy-2-benzyl-4,6-dimethoxycoumaranone) (IX) as reported earlier by Kimura[11] and recently by Lindstedt[12] and Gripenberg[3]. Dehydration of the condensation product with concentrated sulphuric acid yields 4,6-dimethoxyaurone (Xb) identical with an authentic sample.

A parallel series of experiments have been conducted with ω-methoxyresacetophenone and its



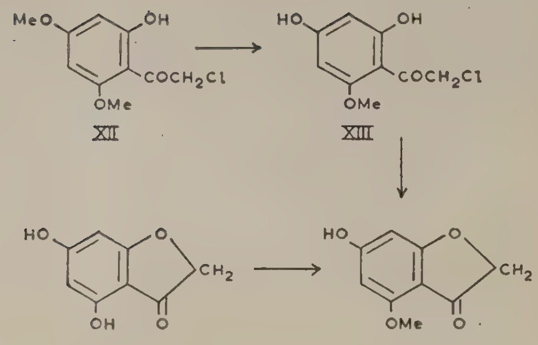
4-methyl ether which yield fisetol[13] and its 4-methyl ether[14] respectively and not the 6-hydroxy and 6-methoxycoumaran-3-ones as reported earlier[10]. These observations reveal an unexpected situation, i.e. the ω-methoxyl is the one which is easily demethylated, and under the strong acidic conditions employed the coumaran-3-one ring closure does not take place and further the ω-hydroxyl group does not undergo change into the bromide. Thus the action of hydrobromic acid offers a convenient and direct method for preparing fisetol derivatives and consequently from them 2-hydroxy-2-benzylcoumaran-3-ones. Earlier 2-hydroxy-2-benzylcoumaran-3-ones were prepared by the action of alkali on dihydroflavonols or by the acid treatment of α-methoxy-chalkones[3,12,15,16] or by the oxidation of 2-benzylcoumaranones[3]. In all these methods, the product is a mixture and the purification is difficult and consequently the yields are not good.

The demethylation reaction with hydrobromic acid was originally intended to differentiate between flavones and flavonols. It is, however, of no value now because more convenient methods are available. For example, ω-methoxyacetophenones obtained from flavonols can be conveniently oxidized to acids by means of iodine with loss of a methoxyl[17].

Methylation of 4,6-dihydroxycoumaran-3-one (XV)

Balakrishna *et al.*[10] carried out the methylation of hydroxycoumaran-3-ones with methyl sulphate and potassium carbonate in dry acetone medium. The products obtained by partial and complete methylation of 4,6-dihydroxycoumaran-3-one (XV) do not, however, agree with the results obtained by other methods. Hence methylation has been reinvestigated and the difference is now found to be due to the interfering effect of acetone solvent which also takes part in the reaction.

Partial methylation of 4,6-dihydroxycoumaran-3-one in acetone solution has been reported earlier to give 6-methyl ether, m.p. 147-8°. This seemed to be an acceptable result since the hydroxyl group in the 4-position was expected to be chelated with carbonyl in the 3-position and hence resistant to methylation. Later Geissman and Hinreiner[18] found that by using diazomethane they could obtain 4-methoxy-6-hydroxycoumaran-3-one (XIV), m.p. 292-3°. This structure was substantiated by synthesis from phloroglucinol monomethyl ether and chloroacetonitrile according to Hoesch reaction[19]. We further confirm this result by another synthesis of this compound. Starting from phloroglucinol trimethyl ether and condensing it with chloroacetyl chloride in the presence of dry aluminium chloride and ether, ω-chloro-

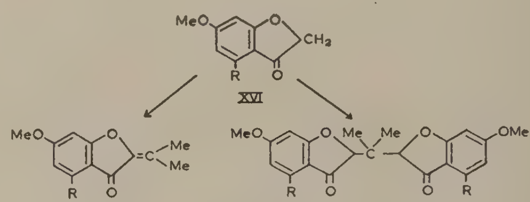


XII XIII XV XIV

2-hydroxy-4,6-dimethoxyacetophenone (XII) has been prepared. Based on analogy with the behaviour of phloroacetophenone di- and trimethyl ethers in partial demethylation[20], this could be converted into 6-methyl ether of ω-chloro-phloroacetophenone (XIII) by heating with anhydrous aluminium chloride in chlorobenzene solution. The product (XIII) has been subsequently cyclized with boiling aqueous potassium acetate to yield 4-methoxy-6-hydroxycoumaran-3-one (XIV) which melts at the same temperature as recorded by Geissman *et al.*[18]. The direct partial methylation of 4,6-dihydroxycoumaran-3-one could also be carried out effectively with one mole of dimethyl sulphate if dioxan-benzene medium is used and the product agrees with the above-mentioned 4-methoxy compound. It may be mentioned here that 4-hydroxy-6-methoxycoumaran-3-one has been synthetically obtained by an unambiguous method by Duncanson *et al.*[21]; its m.p. is 144°.

The complete methylation of 4,6-dihydroxycoumaran-3-one using methyl sulphate in acetone medium was found by Balakrishna *et al.*[10] to give a product, m.p. 101-2°. However, the synthesis by Friedel-Crafts reaction using phloroglucinol trimethyl ether and bromo-acetyl bromide and subsequent cyclization was reported by Friedländer and Schnell[22], Dumat and Tambor[23] as well as by Freudenberg[24] to give 4,6-dimethoxycoumaran-3-one, m.p. 137-8°. Mulholland and Ward[25], who methylated 4,6-dihydroxycoumaran-3-one with methyl sulphate and methanolic alkali, report m.p. 138-9°. In this procedure they reported the simultaneous formation of 5-methyl-4,6-dimethoxycoumaran-3-one and the mixture was separated by chromatography over acid-washed alumina. Under special conditions, the 4,6-dimethoxycoumaran-3-one alone could be obtained.

The difference arising in the acetone-potassium carbonate method seems to be due to complications introduced by the solvent. By using dioxan-benzene medium instead of acetone the dimethyl ether, m.p. 137-8°, could be obtained. Since acetone can condense with the methylene group in the 2-position of the coumaran-3-one structure, it was suspected to be the cause of the low melting point reported by Balakrishna *et al.*[10]. Actually on refluxing authentic 4,6-dimethoxycoumaran-3-one (XVIa) with potassium carbonate and acetone a product melting in the vicinity of 101-2° was obtained. A similar product was obtained when the methylation experiment of Balakrishna *et al.*[10] was repeated. This proved to be a mixture of two compounds, the dimethyl ether, m.p. 137-8°, and a second product, m.p. 212-4°. Separation was effected by making use of the sparing solubility of the latter in ethyl acetate. In order to identify the second compound, the two possible acetone condensation products, (1) 2-isopropylidene-4,6-dimethoxycoumaranone (XVIIa) and (2) 2,2′-*bis*-(4,6-dimethoxycoumaranonyl)-dimethyl methane (XVIIIa), were prepared from 4,6-dimethoxycoumaran-3-one, by methods similar to those reported for analogous derivatives of 6-methoxycoumaran-3-one (XVIb) by Shriner and Anderson[26,27]. There was full agreement in m.p., elemental analyses and ultraviolet absorption spectra only with the *bis* compound (XVIIIa) and not with the isopropylidene derivative (XVIIa).



XVI XVII XVIII

a, R = OCH_3
b, R = H

Parallel series of experiments were performed with 6-methoxycoumaran-3-one (XVIb) which also condensed with acetone in the presence of dry potassium carbonate and the product was again a mixture of the original compound and 2,2′-*bis*-(6-methoxycoumaranonyl)-dimethyl methane[26,27] (XVIIIb).

Chelation in coumaran-3-ones and aurones

The fact that the 4-hydroxyl group in 4,6-dihydroxycoumaran-3-one is not chelated in contrast to the chelated 1-hydroxyl group in xanthones, 5-hydroxyl in flavones and *o*-hydroxyl group in acetophenones seems to be clear from the methylation experiments. This is further supported by the present ultraviolet absorption studies in the case of 4,6-dihydroxycoumaran-3-one and its dimethyl ether in ethanol as well as in cyclohexane (Table 1). Even in a non-hydroxylic solvent there is only a very small hypsochromic shift of the longer wavelength band

TABLE 1 — ULTRAVIOLET ABSORPTION DATA

COMPOUND	λmax. (95% ETHANOL) mμ	DIFF. mμ	λmax. (CYCLOHEXANE) mμ	DIFF. mμ
4,6-Dihydroxycoumaran-3-one (XV)	285	0	270	5
4,6-Dimethoxycoumaran-3-one	285		275	
o-Hydroxyacetophenone[29]	327	22	329	29
o-Methoxyacetophenone[29]	305		300	
4,6-Dihydroxyaurone (Xa)	370	0	*	—
4,6-Dimethoxyaurone (Xb)	370		360	

*Insoluble in cyclohexane.

caused by methylation. On the other hand, there is a hypsochromic shift of 22-29 mμ depending on the solvent when the methylation of the chelated hydroxyl group takes place, e.g. *o*-hydroxy- and *o*-methoxyacetophenones. Similar conclusions have been reached by Farmer *et al.*[28] in their study of 4,7-dihydroxyindanones and 4-hydroxycoumaranones. However, this lack of chelation does not explain why the 4-hydroxyl is preferentially methylated in 4,6-dihydroxycoumaran-3-one. Geissman and Hinreiner[18] have suggested that it is due to the proximity of the 4-hydroxyl group to the carbonyl group which enhances its acidic strength over that of the 6-hydroxyl group sufficiently to allow its preferential methylation.

No clear information about hydrogen bonding is available in the case of hydroxyaurones. Geissman and Harborne[30] have mentioned that methylation of the 4-hydroxyl is very easy in the case of aurones. This is also our experience using dimethyl sulphate and either potassium carbonate or sodium bicarbonate. No partial methylation could be effected in the case of 4,6-dihydroxyaurone (Xa) and the product is a mixture of the dimethyl ether and the unchanged product when only one mole of dimethyl sulphate was employed. Even in the ultraviolet spectra, no hypsochromic shift of the longer wavelength band is caused by methylation (Table 1). It is thus significant to note that in aurones, not only chelation does not exist but also there is no marked difference in acidity of the 4 and 6 hydroxyl groups. An explanation could probably be based on the possibility that conjugation with the side phenyl residue (as found in aurones) reduces the ketonic character of the C=O group and thus its differentiating influence on the phenolic groups.

The fact that all 4-hydroxycoumaran-3-ones and aurones show positive ferric reaction would suggest that there is some chelation. The explanation seems to be that chelation is insignificant in regard to hydrogen bonding between the 4-hydroxyl and 3-carbonyl groups due to steric restriction, but it seems to be possible when a metal bonding is involved. Hence it could be said that hydrogen bonding is not always correctly indicated by the positive ferric reaction.

Experimental procedure

(All ultraviolet absorption data have been recorded in 95 per cent ethanol unless otherwise stated.)

2-*Acetoxy*-4-*methoxy*-ω-*chloroacetophenone* (II) — 2-Hydroxy-4-methoxy-ω-chloroacetophenone[31] (I) (10 g.) was dissolved in acetic anhydride (40 ml.) and two drops of perchloric acid added. The mixture was allowed to stand at room temperature for 30 min. and then poured over ice. The solid (11 g.) that separated crystallized from alcohol as colourless prismatic needles, m.p. 102-3°. (Found: C, 54·70; H, 4·80. $C_{11}H_{11}O_4Cl$ requires C, 54·43; H, 4·53%.) It gave no colour with alcoholic ferric chloride.

2-*Acetyl*-6-*methoxycoumaranone* (III)— A solution of the above ketone (10 g.) in benzene (200 ml.) was refluxed with potassium carbonate (40 g.) for 8 hr; the potassium salts were filtered off, washed with hot benzene and dissolved in water. The reddish brown solution on acidification with dilute sulphuric acid deposited 2-acetyl-6-methoxycoumaranone (III) (4 g.) which crystallized from methanol as very pale yellow needles, m.p. 115-6°. (Found: C, 64·40; H, 5·30. $C_{11}H_{10}O_4$ requires C, 64·07; H, 4·85%.) It gave a deep red colour with alcoholic ferric chloride.

2-*Benzyl*-6-*methoxycoumaranone* (V) — To a solution of sodium (1 g.) in ethyl alcohol (10 ml.) were added 2-acetyl-6-methoxycoumaranone (4 g.) and benzyl chloride (2·5 g.) and the mixture refluxed for 6 hr. Alcohol was then distilled off, the residue treated with water, the solution acidified and the unchanged benzyl chloride steam distilled. The residual solution was extracted with ether and the solvent distilled off when a viscous mass of 2-acetyl-2-benzyl-6-methoxycoumaranone (IV) was left. It was directly refluxed with 5 per cent aqueous sodium carbonate (200 ml.) for 2 hr. The solution was cooled, acidified, extracted with ether and the ether solution dried. The residue (2·4 g.) after removal of ether crystallized first from ethyl acetate-petroleum ether mixture and finally from alcohol as colourless rectangular prisms, m.p. 92-3°. A mixed melting point with an authentic sample[6,32] was undepressed.

6-*Methoxyaurone* (VI) — (i) To a solution of the above coumaranone (V) (1 g.) in acetone (25 ml.) was added powdered potassium permanganate (6 g.) and the mixture left at room temperature for 24 hr. The solvent was distilled off, water added to the resi-

due and sulphur dioxide passed till a colourless solution was obtained which was extracted with ether. The ether residue was dissolved in concentrated sulphuric acid (10 ml.) at 0° and kept at this temperature for 1 hr. It was then poured over ice, extracted with ether and the residue (100 mg.) left after ether evaporation crystallized from methanol yielding colourless rectangular prisms, m.p. 145-6°, alone or admixed with an authentic sample of 6-methoxyaurone[31].

(ii) To a solution of 2-benzyl-6-methoxycoumaranone (V) (1 g.) in carbon tetrachloride (25 ml.) was added N-bromosuccinimide (0·7 g.) and a few crystals of benzoyl peroxide and the mixture refluxed for 2 hr. After cooling, succinimide was filtered off and the solvent from the filtrate distilled off. To the semisolid residue was added alcoholic potash (2 g. in 20 ml.) and the solution refluxed for 2 hr. It was then poured over ice, acidified and extracted with ether. The residue from ether solution gave 6-methoxyaurone (VI) (0·8 g.), m.p. and mixed m.p. with an authentic sample[31], 145-6°.

ω,2-*Dihydroxy*-4,6-*dimethoxyacetophenone* (VIII) — 2-Hydroxy-ω,4,6-trimethoxyacetophenone (VII) (5 g.) was dissolved in acetic acid (55 ml.) and hydrobromic acid (40 per cent, 50 ml.) added to it. The solution was heated on a boiling water bath for 3 hr and poured over ice (400 g.). The solid (1·5 g.) was collected and crystallized twice from methanol when it was obtained as colourless, long, rhombohedral tablets, m.p. 139-40°. (Found: C, 56·13; H, 5·80; OCH_3, 28·83. $C_{10}H_{12}O_5$ requires C, 56·60; H, 5·66; OCH_3, 29·24%.) It gave a red colour with alcoholic ferric chloride and dissolved in 5 per cent aqueous sodium hydroxide; $\lambda_{max.}$, 225, 285 mμ (log ε, 2·85, 3·07 respectively); $\lambda_{min.}$, 240 mμ (log ε, 2·21).

ω-*Hydroxy*-2,4,6-*trimethoxyacetophenone* (XI) — An acetone solution of the above compound (200 mg.) was refluxed with freshly ignited potassium carbonate (4 g.) and dimethyl sulphate (0·3 ml.) until negative ferric reaction (15 hr). After working up as usual, the methyl ether was collected and crystallized from 50 per cent aqueous ethanol when colourless polyhedral prisms separated out, m.p. 185-6°. (Found: C, 59·03; H, 6·20. $C_{11}H_{14}O_5$ requires C, 58·40; H, 6·18%.) $\lambda_{max.}$, 280 mμ (log ε, 3·73); $\lambda_{min.}$, 250 mμ (log ε, 3·31).

2-*Hydroxy*-2-*benzyl*-4,6-*dimethoxycoumaranone* (IX) — To a solution of the ω,2-dihydroxy ketone (VIII) (500 mg.) in 20 per cent aqueous potassium hydroxide (10 ml.) an alcoholic solution of benzaldehyde (400 mg. in 3 ml.) was added. The whole mixture was kept at room temperature for 20 hr. It was acidified, diluted with water and extracted with ether. The ether solution was extracted with aqueous sodium bicarbonate and then washed with water. After drying it over sodium sulphate, the ether solution was evaporated and the residue crystallized from dilute alcohol when 2-hydroxy-2-benzylcoumaranone (IX) formed colourless rectangular plates and prisms, m.p. 171-2°; Gripenberg[3] reported m.p. 171°. (Found: C, 67·50; H, 5·60. $C_{17}H_{16}O_5$ requires C, 68·00; H, 5·33%.) It does not give any colour with alcoholic ferric chloride. $\lambda_{max.}$, 290 mμ (log ε, 4·17); $\lambda_{min.}$, 250 mμ (log ε, 2·23). This product was further characterized by dehydration to 4,6-dimethoxyaurone (Xb) with concentrated sulphuric acid as recorded by Gripenberg[3].

Fisetol-4-*methyl ether* — 2-Hydroxy-ω,4-dimethoxyacetophenone (1 g.) was heated either with aqueous hydrobromic acid (40 per cent, 20 ml.) or with acetic acid (10 ml.) and aqueous hydrobromic acid (40 per cent, 20 ml.) for 3 hr on a boiling water bath. It was filtered through cotton to remove resinous matter and extracted with ether. The solid (0·2 g.) left after ether evaporation crystallized from aqueous methanol as colourless long flat needles and narrow plates, m.p. 127°; Zincke and Eismayer[14] record m.p. 128° for fisetol-4-methyl ether. (Found: C, 59·09; H, 6·20; OCH_3, 15·89. $C_9H_{10}O_4$ requires C, 59·30; H, 5·49; OCH_3, 17·03%.) It gave a red colour with alcoholic ferric chloride. $\lambda_{max.}$, 229, 275, 315 mμ (log ε, 3·94, 4·13, 3·85 respectively); $\lambda_{min.}$, 225, 245, 300 mμ (log ε, 3·89, 2·92, 3·76 respectively).

Fisetol — ω-Methoxyresacetophenone (1 g.) was heated with aqueous hydrobromic acid (40 per cent, 20 ml.) for 3 hr on a boiling water bath. It was worked up as in the previous case. Ether residue (0·15 g.) was crystallized from water containing a few drops of concentrated hydrochloric acid when fisetol separated as colourless needles, m.p. 187-8°. Charlesworth *et al.*[13] report m.p. 189°. It gave a red colour with alcoholic ferric chloride. $\lambda_{max.}$, 232, 275, 313 mμ (log ε, 3·90, 4·02, 3·80 respectively); $\lambda_{min.}$, 225, 250, 300 mμ (log ε, 3·82, 3·27, 3·77 respectively).

4-*Methoxy*-6-*hydroxycoumaran*-3-*one* (XIV): *First method* — 2-Hydroxy-4,6-dimethoxy-ω-chloroacetophenone (XII) (1 g.) in chlorobenzene (10 ml.) was refluxed with aluminium chloride (1 g.) in an oil bath for 1 hr. The solvent was removed under reduced pressure and the residue decomposed with ice and hydrochloric acid (1 : 1; 30 ml.) when a colourless solid (0·8 g.) was obtained. It crystallized from dilute alcohol, m.p. 174-5°. It gave a reddish brown colour with alcoholic ferric chloride.

The ω-chloro-2,4-dihydroxy-6-methoxyacetophenone (0·8 g.) thus obtained was refluxed with aqueous potassium acetate (2 g. in 200 ml.) for 4 hr. The hot solution was filtered, cooled and the solid that separated (0·75 g.) was recrystallized from acetic acid,

when it was obtained as colourless stout rectangular prisms tapering at ends, m.p. 292°. Geissman and Hinreiner[18] record the same m.p. It gave negative ferric reaction. $\lambda_{max.}$, 283 mμ (log ε, 4·42); $\lambda_{min.}$, 243 mμ (log ε, 2·89).

Second method — 4,6-Dihydroxycoumaran-3-one[18] (XV) (0·83 g.) was dissolved in the minimum amount of boiling dioxan, and to this solution an equal volume of dry benzene added. The solution was refluxed with potassium carbonate (3 g.) and dimethyl sulphate (0·5 ml.) for 3 hr. The solvents were removed *in vacuo*, water (50 ml.) added and the mixture extracted with ether. The aqueous solution was acidified and the solid crystallized from acetic acid yielding colourless rectangular prisms, m.p. 292°.

2,2′-*bis*-(4,6-*Dimethoxycoumaranonyl*)-*dimethyl methane* (XVIIIa) — 4,6-Dimethoxycoumaranone (0·97 g.) was dissolved in glacial acetic acid (15 ml.) and acetone (0·25 ml.) and concentrated hydrochloric acid (0·3 ml.) added. The mixture was kept at room temperature for 48 hr with occasional shaking. The solid (0·35 g.) that separated out was filtered and recrystallized from ethyl acetate when colourless aggregates of tiny prisms (m.p. 212-4°) were obtained. (Found: C, 64·12; H, 5·80. $C_{23}H_{24}O_8$ requires C, 64·48; H, 5·65%.) $\lambda_{max.}$, 285 mμ (log ε, 4·67); $\lambda_{min.}$, 240 mμ (log ε, 3·90).

2-*Isopropylidene*-4,6-*dimethoxycoumaranone* (XVIIa) — A solution of 4,6-dimethoxycoumaran-3-one (1 g.), acetone (1·5 ml.) and zinc chloride (0·5 g.) in absolute ethanol (10 ml.) was refluxed for 3 hr. Alcohol was partially distilled off and the solution poured into water. The solid (300 mg.) that separated was filtered and recrystallized twice from ethanol when the isopropylidene derivative formed aggregates of pale yellow rhombohedral prisms, m.p. 168-70°. (Found: C, 65·91; H, 6·40. $C_{13}H_{14}O_4$ requires C, 66·66; H, 6·02%.)

Complete methylation of 4,6-*dihydroxycoumaran*-3-*one with dimethyl sulphate and potassium carbonate*: (a) *Using dioxan and benzene as the medium* — 4,6-Dihydroxycoumaran-3-one (1·66 g.) was dissolved in the minimum amount of boiling dioxan and an equal volume of dry benzene was added along with potassium carbonate (10 g.) and dimethyl sulphate (3 ml.) and the mixture refluxed for 8 hr. The solvents were distilled off *in vacuo*, water (100 ml.) added and the solution extracted with ether. The ether extract was washed with 50 per cent aqueous sodium hydroxide and, after evaporation, the residue was crystallized from benzene when it formed colourless needles, m.p. 137-8° alone or when mixed with 4,6-dimethoxycoumaran-3-one, prepared according to the method of Freudenberg[24]. It gave no colour with alcoholic ferric chloride. $\lambda_{max.}$, 285 mμ (log ε, 4·35); $\lambda_{min.}$, 240 mμ (log ε, 3·80); and ultraviolet absorption in cyclohexane: $\lambda_{max.}$, 275 mμ (log ε, 3·80); $\lambda_{min.}$, 240 mμ (log ε, 2·43).

(b) *Using acetone as the medium* — Solution of 4,6-dihydroxycoumaran-3-one (1·66 g.) in acetone was refluxed with dimethyl sulphate (3 ml.) and ignited potassium carbonate (10 g.) for 30 hr. Acetone was distilled off, water added to the residue and after keeping it for some time, the insoluble product was collected. It was dissolved in warm ethyl acetate and the solution cooled to room temperature. The solid (0·2 g.) was filtered, washed with a little ethyl acetate and recrystallized from it yielding colourless aggregates of tiny prisms, m.p. 212-4°. Mixed m.p. with an authentic sample of 2,2′-*bis*-(4,6-dimethoxycoumaranonyl)-dimethyl methane (XVIIIa) was undepressed.

The mother liquor was concentrated partially, and petroleum ether added until no more resin separated. The clear solution was decanted and cooled in the refrigerator when a colourless solid (1·1 g.), m.p. 137-8°, was obtained. It was found to be identical with 4,6-dimethoxycoumaran-3-one.

Reaction of 4,6-*dimethoxycoumaran*-3-*one with potassium carbonate* — An acetone solution of 4,6-dimethoxycoumaran-3-one (1 g.) was refluxed with anhydrous potassium carbonate (5 g.) for 24 hr. After the removal of acetone, water was added to the residual mixture. The solid was collected and dissolved in the minimum amount of boiling ethyl acetate. The first crop (200 mg.) was a colourless substance, m.p. 212-4°. The mixed m.p. with an authentic sample of 2,2′-*bis*-(4,6-dimethoxycoumaranonyl)-dimethyl methane was undepressed. The mother liquor was concentrated and allowed to cool. The solid that separated (0·6 g.) was found identical with the starting material.

The above experiment was repeated using benzene as the solvent and the substance was recovered unchanged.

Reaction of 6-*methoxycoumaran*-3-*one with potassium carbonate and acetone* — 6-Methoxycoumaran-3-one[31] (1 g.) in dry acetone solution was refluxed with ignited potassium carbonate (5 g.) for 40 hr and worked up as in the previous case. The fraction sparingly soluble in ethyl acetate was recrystallized from the same solvent, yielding colourless aggregates of thin plates (250 mg.), m.p. 209-10°. This was found to be identical with 2,2′-*bis*-(6-methoxycoumaranonyl)-dimethyl methane, prepared according to the method of Shriner and Anderson[26,27]. The ethyl acetate mother liquor yielded the unchanged coumaranone, m.p. 119-20°.

4,6-*Dihydroxyaurone* (Xa) — 4,6-Dihydroxycoumaran-3-one (1 g.) was dissolved in aqueous potassium

hydroxide (20 per cent, 20 ml.), benzaldehyde (1 g.) in alcohol (20 ml.) added and the clear red solution allowed to stand at room temperature for 20 hr. After acidification, a fine yellow powder (0·75 g.) separated. It was first crystallized from dilute alcohol and then from ethyl acetate-petroleum ether when 4,6-dihydroxyaurone was obtained as tiny yellow prisms, m.p. 252-3°. (Found: C, 71·12; H, 4·26. $C_{15}H_{10}O_4$ requires C, 70·86; H, 3·96%.) It gave a brown colour with alcoholic ferric chloride and with concentrated sulphuric acid, an orange colour. $\lambda_{max.}$, 315, 370 mμ (log ε, 4·33, 4·36 respectively); $\lambda_{min.}$, 245, 325 mμ (log ε, 4·12, 4·30 respectively).

4,6-Dimethoxyaurone (Xb)— 4,6-Dihydroxyaurone (1 g.) was refluxed in dry acetone (75 ml.) with potassium carbonate (2 g.) and dimethyl sulphate (0·8 ml.) for 10 hr. Acetone was removed and water (100 ml.) added; the product (0·8 g.) crystallized from alcohol as colourless rectangular tablets, m.p. 157° alone or when mixed with the sample prepared from 4,6-dimethoxycoumaran-3-one by the acid method; $\lambda_{max.}$, in 95 per cent ethanol, 370 mμ (log ε, 4·35), $\lambda_{min.}$, 270 mμ (log ε, 3·45); $\lambda_{max.}$ in cyclohexane, 300, 360 mμ (log ε, 3·76, 3·89 respectively), $\lambda_{min.}$, 270, 320 mμ (log ε, 3·36, 3·74 respectively).

When the above methylation was effected using only one mole of dimethyl sulphate either in the presence of potassium carbonate or sodium bicarbonate, the product was a mixture of the unchanged compound and 4,6-dimethoxyaurone.

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Paper Chromatographic Analysis of Acids (Horizontal Migration Method): Part VII*

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The circular R_f values of inorganic anions, viz. arsenate, chromate, ferricyanide, ferrocyanide, halides, oxy-halides, nitrate, sulphate and thiocyanate, have been determined in six solvents, namely *n*-butanol-ammonia, *n*-butanol-pyridine-water-ammonia, ethanol-ammonia-water, 2,6-lutidine-water, collidine-water and *n*-propanol-ammonia; the last three solvents have been used for the first time in the chromatography of inorganic anions. The amount of water present in 2,6-lutidine influences the R_f value and 70 per cent aqueous lutidine has been found to give satisfactory results. Collidine saturated with water is useful in the separation of oxy-halides. The effect of varying *p*H on the R_f values of chloride and nitrate ions is not significant. The study of the chromatographic behaviour of various chloride salts has shown that the presence of ammonium and lithium cations increases the R_f value of chloride ion relative to that of sodium or potassium ions.

IN the earlier communications[1-4], the suitability of Rutter's modification of paper partition chromatography was examined with reference to organic and amino acids. The studies were extended to inorganic anions and the results are reported in this communication.

Experimental procedure

The procedure followed was essentially same as that described earlier[5]. Unwashed Whatman No. 1 filter paper discs (diam. 15 cm.) were used, unless otherwise stated. All the experiments were carried out in an electrically controlled incubator either at 30° or 35° ± 1°C.

Solvents — *n*-Butanol, *n*-propanol, pyridine, ammonia (*d*, 0·96) of E. Merck, collidine and 2,6-lutidine (meant for chromatographic work) of Fluka, and ammonia (*d*, 0·88) of May & Baker were used. The ratios given for solvent mixtures were by volume.

In the case of *n*-butanol saturated with 1·5*N* ammonia, collidine saturated with water and *n*-butanol-pyridine-water-ammonia (*d*, 0·96) (80:40:77:8) mixtures, the organic-rich phases were used as solvents.

Solutes — About 0·2*M* solutions of sodium or potassium salts of the respective anions were used. Hydrochloric acid and nitric acid of 0·2*N* strength were adjusted with a few ml. of dilute sodium hydroxide solution to required *p*H values and used to study the influence of *p*H on the R_f value of an anion.

Reagents — Special reagents suggested for the identification of inorganic ions in *Chromatographic Methods of Inorganic Analysis* by Pollard and McOmie[6], and in *Chromatography* by Lederer and Lederer[7] were employed in this study.

For the detection of halides (with the exception of fluoride) and oxyacids of halogens, the Duncan and Porteous reagent[8] was found useful. Fluoride zone was identified with the zirconium-alizarin reagent.

Results and discussion

In order to determine the movement of different anions on filter paper discs, six alkaline solvent mixtures were tried. These mixtures included the three that were tried and found useful in conventional methods, namely *n*-butanol saturated with 1·5*N* ammonia, *n*-butanol-pyridine-water-ammonia and 95 per cent ethanol-ammonia-water. Among the others the following three solvents were found good: 2,6-lutidine-water, collidine-water and *n*-propanol-ammonia solvents.

With a view to finding out the optimum concentration of water in the 2,6-lutidine-water solvent system, the R_f values of halides were determined with 75, 70 and 65 per cent aqueous lutidine mixtures. Besides, lutidine-ammonia system was also examined.

The results presented in Table 1 indicate that the R_f values of each halide ion increased as the water

*This work formed a part of the Ph.D. thesis submitted by the author to the Lucknow University.

content of lutidine solvent increased. However, it was observed that the definition of the zones as well as the degree of resolution decreased when the water content in the solvent was more than 35 per cent. In further studies 70 per cent aqueous lutidine was used as this composition was found to be the best.

Between the lutidine-water and lutidine-ammonia solvents, the latter gave diffused zones with halides. So, the presence of ammonia in this solvent did not advantageously influence the movement of anions.

The chromatographic behaviour of halides and halogen oxyacids was studied in *n*-propanol-ammonia solvent system and the R_f values are recorded in Table 2. This solvent system appeared to be very sensitive to changes in the batch of ammonia used. Hence the average of three R_f values was taken into account. It might be mentioned here that *n*-propanol-ammonia solvent system was found suitable for the analysis of aliphatic acids[4].

One interesting observation was the reversal in the order of R_f values of chloride and bromide ions in *n*-propanol-ammonia (70: 30) solvent (Table 2). A similar reversal in the usual order of R_f values of chloride, bromide and iodide was reported by Lederer[7] with butanol shaken with 20 per cent aqueous acetic acid mixture. The halide zones formed with either of these solvents were well-defined, but those obtained for halogen-oxyacids were quite diffused. Recently Cohen and Lederer[7] reported similar values for chlorate and iodate ions using ethanol-water-15N ammonia solvent.

TABLE 1 — EFFECT OF WATER IN 2, 6-LUTIDINE SOLVENT ON R_f VALUES OF HALIDES

(*Temp.*, 30°C.)

Anion	Lutidine-water			Lutidine-ammonia (d,0·88)-water
	75: 25	70: 30	65: 35	70: 30: 20
Fluoride	0·25	0·32	0·43	0·43
Chloride	0·40	0·48	0·56	0·56
Bromide	0·50	0·61	0·69	0·67
Iodide	0·72	0·75	0·83	0·86

Besides the lutidine-water and *n*-propanol-ammonia mixtures, collidine saturated with water was found quite useful, especially with regard to halogen oxyacids. The zones obtained with this solvent were quite compact.

In view of the usefulness of the above six solvents, the R_f values of various anions were determined and the results are given in Table 3.

Out of these six solvents, the utility of the three newly introduced solvent systems has been already discussed. From the rest *n*-butanol-pyridine-water-ammonia solvent was prepared using ammonia of 0·96 density instead of 0·88, as reported earlier by Pollard[6]. Due to this reason the original proportion of Pollard, viz. 80: 40: 80: 5, was altered to 80: 40: 77: 8 in these studies. The new solvent, however, gave in some cases diffused zones and streaks, which were not reported earlier by Pollard.

n-Butanol-1·5N ammonia solvent of Lederer[7] gave discrete zones. In the present study the filter paper discs were pre-washed with the mobile phase before use. The results obtained were in complete agreement with those of Lederer.

Long *et al.*[9] found that 95 per cent ethanol-ammonia-water (80: 4: 16) solvent was good when used with Whatman No. 54 filter paper. But this solvent gave very high R_f values for acids on Whatman No. 1 filter paper discs (Table 3). It might be pointed out in this connection that sulphate anion gave a high R_f value, namely 0·77, when all other solvents gave low values for this anion.

As the pH of the solution affects the R_f values of organic acids[2], the R_f values of chloride and nitrate ions at different pH levels were determined in three solvents (Figs. 1 and 2). The results, except for a few values, would indicate that the variation of R_f value with pH is not much.

With a view to determining the influence of various cations on chloride ion, different chloride salts were chromatographed and the R_f values are given in Table 4. The R_f values of chloride ion varied from 0·55 to 0·63. It was noted that the presence of ammonium and lithium cations increased the R_f

TABLE 2 — R_f VALUES OF HALIDES AND HALOGEN OXYACIDS IN *n*-PROPANOL-AMMONIA SOLVENT MIXTURE

(*Temp.*, 35°C.)

Anion	*n*-Propanol-ammonia (d, 0·88) (80: 20): R_f values				*n*-Propanol-ammonia (d, 0·88) (70: 30): R_f values			
	I	II	III	Av.	I	II	III	Av.
Chloride	0·42	0·42	0·50	0·45	0·71	0·68	0·76	0·72
Bromide	0·46	0·49	0·54	0·50	0·60	0·64	0·59	0·61
Iodide	0·57	0·61	0·63	0·60	0·72	0·76	0·77	0·75
Chlorate	0·52	0·44	—	0·48	0·77	0·68	—	0·72
Bromate	0·37	0·37	—	0·37	0·62	0·55	—	0·59
Iodate	0·00	0·00	—	0·00	0·52	0·40	—	0·46

TABLE 3 — MOVEMENT OF ANIONS IN DIFFERENT SOLVENT SYSTEMS

Anion	A*	B†	C†	D†	E*	F*
Fluoride	0·06	—	0·32	0·27	—	0·72
Chloride	0·18	0·36	0·47	0·34	0·45	0·83
Bromide	0·20	0·41	0·61	0·43	0·50	0·76
Iodide	0·26	0·64	0·75	0·67	0·60	0·92
Chlorate	0·31	0·54	0·69	0·62	0·48	0·87
Bromate	0·18	0·35	0·49	0·37	0·37	0·72
Iodate	0·04	0·26 (diffused)	0·22	0·15	0·00	0·58
Thiocyanate	0·50	0·69	0·82	—	—	—
Nitrate	0·28	0·50	0·71	0·50	0·59	0·87 (diffused)
Ferricyanide	0·00	0·34	—	—	—	—
Ferrocyanide	0·00	0·30 (streak)	—	—	—	—
Sulphate	0·00	Streak	0·00	—	—	0·77
Chromate	—	0·28	0·00	0·00	—	0·19
Arsenate	0·00	0·34 (streak)	—	—	—	—

A, *n*-butanol-1·5*N* ammonia; B, *n*-butanol-pyridine-water-ammonia (*d*, 0·96), 80: 40: 77: 8; C, 2,6-lutidine-water, 70: 30; C, collidine-water; E, *n*-propanol-ammonia (*d*, 0·88), 80: 20; F, 95% ethanol-ammonia (*d*, 0·88)-water, 80: 4: 16.
*Temp., 35°C.
†Temp., 30°C.

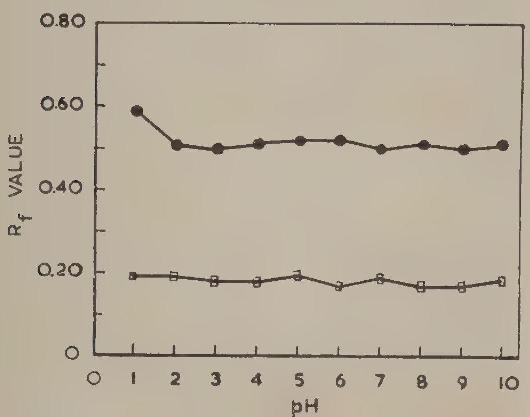

Fig. 1 — Effect of *p*H of sodium chloride solution on the movement of chloride ion [•—•, *n*-butanol-1·5*N* ammonia, temp., 35°C.; □—□, 2,6-lutidine-water (70:30), temp., 30°C.]

values of chloride ion relative to that of sodium and potassium cations.

The results presented in Table 4 and Figs. 1 and 2 suggest that the concentration and the nature of the cation present do not much influence the R_f value of an anion under the present experimental conditions.

It is, however, still not quite clear whether the anions will move as they are, as free acids or in combination with the base present in the solvent. Westall[10], however, assumes the formation of sodium phenate ions in the separation of sodium ion from chloride ion using phenol solvent.

TABLE 4 — R_f VALUES OF CHLORIDES IN 2,6-LUTIDINE-WATER (65 : 35) SOLVENT SYSTEM

(*Temp.*, 30°C.)

Salt	R_f value	
	Cation	Anion
Lithium chloride	—	0·63
Ammonium chloride	0·22	0·62
Sodium chloride	0·32	0·55
Potassium chloride	0·35	0·55
Cadmium chloride	—	0·59
Copper chloride	0·35	0·62
Nickel chloride	—	0·61
Manganese chloride	—	0·62
Chromium chloride	—	0·59
Barium chloride	0·08	0·57
Strontium chloride	—	0·58

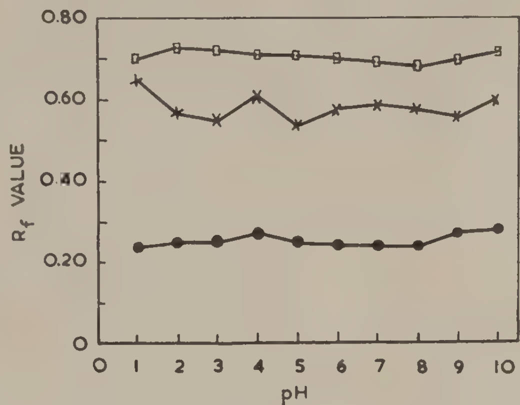

Fig. 2 — Effect of *p*H of sodium nitrate solution on the movement of nitrate ion [•—•, *n*-butanol-1·5*N* ammonia, temp., 35°C.; □—□, 2,6-lutidine-water (70: 30), temp., 30°C.; ×—×, *n*-propanol-ammonia (*d*, 0·88) (80: 20); temp., 35°C.]

Acknowledgement

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Letters to the Editor

TRACE ELEMENTS IN MANGANESE ORES OF VISAKHAPATNAM DISTRICT

Spectrographic examination of the trace elements in manganese ores of Visakhapatnam district, Andhra State, has shown that the manganese ores in this area are original sediments and the trace elements present in them are extraneous, being introduced by intrusive pegmatites or the contaminations of the other para-gneisses associated with the ore body.

A QUALITATIVE SPECTROGRAPHIC EXAMINATION OF manganese ores of Visakhapatnam district, Andhra State, is recorded here for the first time. The data throw light on the genetic nature of the ores of the area.

With a view to detecting qualitatively the minor and trace elements present in manganese ores, a few samples were spectrographically analysed using the E_1 large quartz spectrograph supplied by Adams Hilger & Co., England. A d.c. carbon arc was used with 4 to 8 amp. current and spec.-pure carbon electrodes were used in the experiments.

Representative samples of finely powdered ore material were put into the crater made in the carbon electrode. The spectra were photographed in the region between 2300 and 5200 A. on Ilford special rapid plates. The spectra of ore, the spectra of Raies Ultimes (R.U.) powder and iron were juxtaposed. The plates were developed and fixed in the usual manner. A study of the plates indicated the existence of trace elements, which differ from sample to sample.

As the carbon rods contain boron in negligible amounts, it is possible to detect boron in the ores by the occurrence of two lines 2497·73-2496·78 A. The ores show the presence of silicon, aluminium, calcium, nickel, copper, cobalt, magnesium and possibly lead.

The results are given in Table 1 and typical spectra of ore samples with the markings for the R.U. lines of observed trace elements are reproduced in Fig. 1. The occurrence of the trace elements in the ores can be accounted for as detailed below.

Calcium, magnesium and aluminium — The presence of calcium and magnesium, in small amounts, in the ores is known. Aluminium is sometimes found in estimable quantity, especially in Koduru and

TABLE 1 — SPECTROGRAPHIC ANALYSIS OF THE ORES

ELEMENT	Sample No.: 05 Mineral: VREDENBURGITE	04 PSILOMELANE	01 BRAUNITE	03 PSILOMELANE	8 PSILOMELANE
Calcium	P	—	P	P	—
Barium	P	P	P	—	Tr
Magnesium	P	—	P	Tr	P
Zinc	P	—	—	—	—
Nickel	P	Tr	P	Tr	—
Cobalt	P	P	P	P	Tr
Boron	P	—	Tr	—	—
Aluminium	—	P	—	P	—
Titanium	P	P	—	P	—
Copper	—	—	P	—	Tr

P, present; Tr, trace.

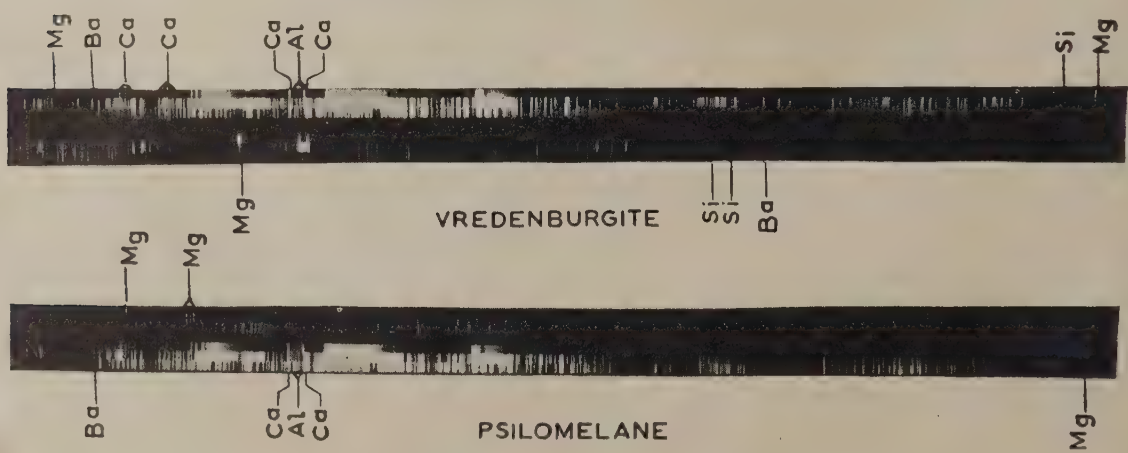

FIG. 1 — SPECTRA OF SAMPLES OF VREDENBURGITE AND PSILOMELANE

Garbham ores. The occurrence of the ore in association with argillaceous, arenaceous and calcareous rocks explains the presence of these trace elements in the ores. Calcium is less abundant than magnesium or aluminium in the ores. The presence of calcium, magnesium and aluminium in these ores can be traced to the associated formations.

Zinc and copper — The presence of zinc has been observed in vredenburgite and of copper in braunite. The presence of copper and zinc in these ores may be due to adsorption of these elements by the ore. The source of copper and zinc may be traced to the 'oxydate sediments' rich in manganese.

Barium — Barium is found in most of the ores from trace quantities to a considerable amount, as much as 11 per cent in the form of BaO. The source of barium in manganese ores of the Visakhapatnam district was considered by Fermor[1] as extraneous and having been introduced by circulating waters. Noll[2] concluded that barium is enriched in rocks carrying potash feldspars. Shimer[3] found that barium predominates in the feldspars of granite and pegmatites. In a similar way the pegmatites in the area, containing apatites and potash feldspars, may be the source of barium. On alteration of these, barium will be separated and introduced into manganese. Also, according to Ramdohr[4] psilomelane contains barium in crystal lattice.

Boron — Boron has been detected in vredenburgite and braunite. Wasserstein[5] considered that the presence of boron in manganese ores is significant in that it leads to the definite identification of braunite. Boron is also noted in vredenburgite from Koduru mines. Boron might have been there along with manganese in the original sediments or it might have been introduced due to the intrusion of pegmatites and granites. The latter source has more direct evidence as the pegmatites in the area contain minerals like tourmaline and topaz.

Titanium — It is possible that small amounts of titanium are incorporated in silicon-oxygen tetrahedra. Recent crystal chemical studies[6] have revealed that the Si-Ti diadochy might be of less importance for the manner of occurrence of titanium and that the bulk of titanium would replace aluminium, ferric iron and magnesium in minerals. The latter may be true in the case of vredenburgite. The small amounts of titanium present in these ores are concentrated in some places probably due to leaching action.

Cobalt and nickel — Cobalt is present in psilomelane, braunite and vredenburgite. Nickel occurs in traces in psilomelane; it is also found in braunite and vredenburgite. Cobalt and nickel are pronouncedly siderophile and have weak affinity for oxygen. Cobalt is less siderophile and this is probably the reason for its minute occurrence with manganese ores. The lithophile tendency of nickel and cobalt explains their occurrence in braunite. In general, cobalt and nickel occur as sulphide segregations of pyrrhotite-pentlandite group. In the Visakhapatnam district, there are sulphide segregations containing pyrite, although minute, and they are known to occur in Ramabhadrapuram. The pyrite can accommodate considerable cobalt and also a little nickel. During the process of alteration of these pyrites, nickel and cobalt are liberated forming colloidal hydroxides which might have been deposited in manganiferous oxidates.

It is clear from the above that the manganese ores of the area are original sediments and some of the trace elements present in them are extraneous being introduced by intrusive pegmatites or the contaminations of the other para-gneisses associated with the ore body.

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CHARACTER OF THE GREAT ICE AGE IN THE KASHMIR HIMALAYAS

On the basis of studies carried out to find whether the series of fluvioglacial deposits in the hills near Jammu, formed as a result of Pleistocene glaciation in the Batote hills and the higher ranges northwards, throw some light on the extent, spacing and intensity of glacial cycles of the Ice Age in the Kashmir Himalayas, it has been concluded that the character of the Ice Age in these mountains is almost identical with that in the Alps.

Subsequent to the discovery of Pleistocene glaciation in the Batote (Jammu) hills[1] and its indications in the upper Siwalik rocks, viz. the Pinjor conglomerate and the boulder conglomerate exposed in the neighbourhood of Jammu[2], it was shown that these as well as the post-Siwalik deposits in the area are not fluviatile but fluvioglacial in origin, and

have been laid down during the Great Ice Age[3]. The post-Siwalik deposits included in this category are: (1) the pebble and boulder beds and (2) the brick-red pebble beds, both soft and friable deposits. In a recent note on the fluvioglacial deposits in the outer Himalayas, the author placed both these beds in one and the same group on lithological grounds. But considering the brick-red colour of its upper portion (30-50 ft thick), separated from the underlying beds by limonitic clays, it is advisable to split it up into two parts. These brick-red beds directly form the basement of Jammu town and may be designated as the *Jammu beds*.

In the communications referred to above, the Pinjor conglomerate and sandstone have been stated to have been formed mostly in the post-Gunz interval, the boulder conglomerate in the post-Mindel period, and the pebble and boulder beds in the post-Riss interval. With the division of the last deposit into two parts, the Jammu beds were very likely formed in the period immediately following the Würm cycle.

From the occurrence of the above-mentioned four groups of deposits in the outer Jammu hills, separated from each other by unconformities or limonitic clays, and from the fact that the first two deposits are hard and compact and the other two soft and incoherent, some interesting inferences can be drawn about the character of the Great Ice Age inasmuch as it affected the Kashmir Himalayas. These are:

(1) It was multiple in character, consisting of four glacial cycles, separated by interglacial periods, as in other parts of the world.

(2) It shows bipartitioning or division into two large phases, the first phase consisting of the Gunz and the Mindel cycles and the interval between them and the other of the Riss and Würm cycles and the Riss-Würm interval, the two having been separated from each other by the longest interglacial period, viz. Mindel-Riss period. The bipartitioning is evident from the fact that, whereas the first two deposits in the area are compact, the other two are incoherent, showing that before the pebble and boulder beds belonging to the Riss cycle and the interval following it began to be formed, the previous two deposits, viz. the Pinjor conglomerate and the boulder conglomerate, had sufficient time to acquire a fairly high degree of compactness. This particular character of the Great Ice Age has also been brought out by Heim in the Alps, where he has traced four groups of glacial deposits, viz. the Older Deckenschotter, the Younger Deckenschotter, the High Terrace Gravels and the Low Terrace Gravels[4]. These correspond to the four deposits in the Jammu area.

(3) The Mindel cycle was most intense as during this period the snowfields on the mountains descended to the latitude of Batote hills, near Jammu, from where the melting glaciers were able to erode and transport abundant boulders of the Murree sandstone, contained in the boulder conglomerate. The boulder conglomerate is also the thickest of the fluvioglacial deposits indicating the great thickness of the snowfields during the Mindel stage.

(4) The post-Würm period appears to have been the most arid and hot of the interglacial periods, and its hot and arid climate gave rise to the brick-red colour of the Jammu beds. The present hot and arid climate of the region is probably a continuation of the post-Würm climatic conditions.

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CHROMATOGRAPHIC SEPARATION OF ZIRCONIUM & HAFNIUM OXYCHLORIDES ON SILICA GEL

A natural mixture of hydrated zirconium and hafnium oxychlorides, obtained by alkali fusion of Indian zircon followed by crystallization from hydrochloric acid, has been used directly in an acidic methanolic solution for frontal separation of the two oxychlorides over activated silica gel. Three fractions have been collected: (i) Hf/Zr wt ratio $\leqslant$0·01 per cent, (ii) Hf/Zr c. 0·5 per cent and (iii) Hf/Zr >20 per cent, with average yields of 70-75, 25-20 and 5 per cent of input in terms of oxide equivalents.

HANSEN AND CO-WORKERS[1-3] REPORTED A METHOD OF separating zirconium from hafnium based on the preferential adsorption of the latter by silica gel from a solution of the anhydrous tetrachlorides in dry methanol. An economic evaluation of the method was made by Millard and Maitland[4]. By converting the hafnia recovered from the columns to the anhydrous chloride and recycling into the process, Beyer *et al.*[5] succeeded in preparing a 90 per cent concentrate of hafnium oxide.

The somewhat difficult preparation and handling of the anhydrous tetrachlorides, and their reactivity to organic solvents led us to seek methods of separation of zirconium from hafnium by starting from the

oxychloride, which is more stable and easy to prepare. In this connection, an observation was first made by T. P. Sarma* of this laboratory (in early 1957) that a difference in adsorption on silica gel might take place even of the oxychlorides in methanol. The oxychlorides, like the tetrachlorides, have a good solubility in this solvent.

Operating with zirconium-hafnium oxychloride prepared from Indian zircon ($Hf/Zr = 3{\cdot}0 \pm 0{\cdot}15$ per cent) under carefully worked out conditions and in columns up to 70 mm. in diam. packed with activated silica gel, we have found that the following fractions of oxide equivalents could be isolated:

(1) Pure zirconia: 70-75 per cent of input with $\leqslant 0{\cdot}01$ per cent Hf/Zr

(2) Low-hafnia zirconia: 25-20 per cent of input with about 0·5 per cent Hf/Zr

(3) High-hafnia zirconia: 5 per cent of input with >20 per cent Hf/Zr

Five lb. of active gel yield a pound (or more) of pure zirconia. The gel and the methanol recovered from the fractions could be re-used after suitable treatment. The high-hafnia fraction in methanol, which could be recycled to yield a still higher concentrate, was found to be a suitable material for the preparation of pure hafnium oxide[6].

Hansen's method starts with the tetrachloride and recovers the separated elements as oxychlorides. For higher concentration of hafnium, the recycling requires recovery of the oxide by ignition of the hydroxide or oxychloride and its chlorination to get back the anhydrous tetrachloride. The direct use of the oxychloride has obvious advantages and does not decrease the yields of pure (reactor grade) zirconia.

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SYNTHESES OF CARTHAMIDIN & DIHYDROWOGONIN

A new synthesis of carthamidin has been achieved by subjecting 5,8-dimethoxy-7,4′-dibenzyloxy flavanone to oxidative demethylation and subsequent catalytic hydrogenation. An improved method of preparing dihydrowogonin is by the demethylation of 5,8-dimethoxy-7-hydroxy flavanone with aluminium chloride in acetonitrile.

Aluminium chloride in dry ether has been shown to be a convenient reagent for the selective demethylation of the 5-methoxyl of flavanones and it has been used for the synthesis of naturally occurring flavanone, dihydrowogonin (5,7-dihydroxy-8-methoxy flavanone) from 5,8-dimethoxy-7-hydroxy flavanone by Aiyar *et al.*[1]. Chopin and Chadenson[2] have mentioned that they have not found it satisfactory and that the demethylation was incomplete. They, therefore, employed a mixture of benzene and ether. Here also they obtained a mixture of dihydrowogonin and 5,7,8-trihydroxy flavanone but it could be easily separated. We have re-examined the original method of Aiyar *et al.*[1] and found that the earlier report is correct and further that the demethylation of the 5-position goes to completion whenever there is the formation of a liquid or semi-solid aluminium chloride complex; otherwise it is incomplete and mixtures are formed. The exact conditions necessary for such complex formation could not be defined. We have, therefore, looked for better methods; the use of anhydrous acetonitrile as solvent serves the purpose very well and demethylation of the 5-position alone takes place to completion, thus providing an improved and more convenient method of synthesis.

The synthesis of 5,7,8-trihydroxy flavanone and 5,7,8,4′-tetrahydroxy flavanone (carthamidin) was reported by Narasimhachari *et al.*[3] using aluminium chloride and benzene for the demethylation of the corresponding tri- and tetramethyl ethers. Similarly the synthesis of 5,6,7-isomers was also reported by them. Arthur[4] has employed the same reagent for demethylating matteucinol (5,7-dihydroxy-4′-methoxy-6,8-dimethyl flavanone) into ferrerol (5,7,4′-trihydroxy-6,8-dimethyl flavanone) and later Zemplen *et al.*[5] for the demethylation of 6,4′-dihydroxy-5,7-dimethoxy flavanone into isocarthamidin. It has been our general experience that this combination (aluminium chloride and benzene) does not always give satisfactory and consistent results in flavanones. A more unequivocal method for the synthesis of carthamidin seemed to be needed. After exploring a number of alternative methods a convenient method has now been discovered. It consists in the prepara-

tion of 7,4′-dibenzyloxy-5,8-dimethoxy flavanone and subjecting it to oxidative demethylation with nitric acid. The resulting dibenzyloxy-5,8-quino flavanone could be simultaneously reduced and debenzylated by means of hydrogen and palladium-charcoal catalyst. 5,7,8,4′-Tetrahydroxy flavanone or carthamidin is thus obtained in satisfactory yields. The lower member of the carthamidin series is 5,7,8-trihydroxy flavanone and it is more easy to prepare. We find that the demethylation of 5,8-dimethoxy-7-hydroxy flavanone with aluminium chloride in dioxan solution[6] is the most suitable method for its preparation and the product comes without any admixture.

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NEW AMIDES & THEIR REDUCTION TO AMINES

The preparation of three new amides, viz. N(β-3,4-dimethoxyphenethyl)-*cis*-3-methoxycyclohexanecarboxylic acidamide (I), N(β-3,4-dimethoxyphenethyl)-*cis*-3-hydroxycyclohexanecarboxylic acidamide (II) and N-ethyl-*p*-methoxybenzanilide (III), and their reduction to the corresponding amines with lithium aluminium hydride are reported.

THE USE OF LITHIUM ALUMINIUM HYDRIDE IN THE reduction of amides to amines was first reported by Ehrlich[1] who reduced an anilide to a secondary amine in tetrahydrofuran. Later a number of workers[2-6] have reported the use of lithium aluminium hydride in the reduction of amides to amines. In the present communication the preparation of three new amides, viz. N(β-3,4-dimethoxyphenethyl)-*cis*-3-methoxycyclohexanecarboxylic acidamide (I), N(β-3,4-dimethoxyphenethyl)-*cis*-3-hydroxycyclohexanecarboxylic acidamide (II) and N-ethyl-*p*-methoxybenzanilide (III), and their reduction to the corresponding amines with lithium aluminium hydride is reported.

N(β-3,4-Dimethoxyphenethyl)-cis-3-methoxycyclohexanecarboxylic acidamide (I) — Methyl-*cis*-3-methoxycyclohexanecarboxylate (1·7 g.) prepared according to the method of Noyce and Denney[7] and homoveratrylamine (1·8 g.) were mixed together and heated at 190-200°C. for 4 hr and cooled. The product was recrystallized from benzene; yield 2·5 g. (78 per cent); m.p. 94-5°. (Found: C, 67·35; H, 8·43; N, 4·51. $C_{18}H_{27}NO_4$ requires C, 67·30; H, 8·41; N, 4·36%.)

N(Cis-3-methoxyhexahydrobenzyl)-β-3,4-dimethoxyphenethylamine — The amide (I) (0·5 g.) in ether (25 ml.) was added dropwise to a solution of lithium aluminium hydride (1·5 g.) in ether (50 ml.) in a three-necked flask (100 ml.) fitted with a mercury sealed stirrer and a condenser with $CaCl_2$ guard tube. The reaction mixture was allowed to reflux for 24 hr, cooled in an ice bath and the excess lithium aluminium hydride destroyed by the addition of ice-cold water with vigorous shaking. The precipitated inorganic hydroxides were destroyed by the dropwise addition of ice-cold 10 per cent sulphuric acid. The mixture was extracted with ether to remove unchanged aldehyde and then basified with 20 per cent sodium hydroxide solution and extracted thoroughly with ether. The ether extract was dried over anhydrous sodium sulphate and the solvent removed; yield 0·35 g. (78 per cent); b.p. 160-65°/0·5 mm.; $[n]_D^{22}$, 1·5270. (Found: C, 70·35; H, 9·79; N, 4·67. $C_{18}H_{29}NO_3$ requires C, 70·35; H, 9·44; N, 4·56%.)

N(β-3,4-Dimethoxyphenethyl)-cis-3-hydroxycyclohexanecarboxylic acidamide (II) — *Cis*-3-hydroxycyclohexanecarboxylic acid (1·45 g.), prepared according to the method of Noyce and Denney[8], and homoveratrylamine (1·81 g.) were mixed together and heated at 180-90° for 1 hr and cooled. The product was recrystallized from benzene; yield 2·5 g. (84 per cent); m.p. 103-4°. (Found: C, 66·99; H, 8·45; N, 4·62. $C_{17}H_{25}NO_4$ requires C, 66·44; H, 8·14; N, 4·56%.)

N(Cis-3-hydroxyhexahydrobenzyl)-β-3,4-dimethoxyphenethylamine — The amide (II) (0·5 g.) was reduced with lithium aluminium hydride (1·5 g.) in tetrahydrofuran (20 ml.) in a three-necked flask (250 ml.) as before. In this case the mixture was refluxed for 35 hr and the excess lithium aluminium hydride destroyed by the dropwise addition of aqueous tetrahydrofuran and the solution filtered. The residue was washed thoroughly with warm tetrahydrofuran and the mixed tetrahydrofuran extracts dried over anhydrous sodium sulphate and the solvent removed; yield 0·28 g. (59 per cent); b.p. 170-75°/0·5 mm.; $[n]_D^{22}$, 1·5295. (Found: C, 69·35; H, 9·30; N, 4·72. $C_{17}H_{27}NO_3$ requires C, 69·60; H, 9·25; N, 4·77%.)

N-Ethyl-p-methoxybenzanilide (III) — Anisoyl chloride (6 g.) in ether (50 ml.) was added to a solution of ethyl aniline (17 g.) in ether (100 ml.). Ethyl aniline

hydrochloride formed was removed by filtration. To the filtrate dilute hydrochloric acid was added and the mixture extracted with ether. The ether extract was dried over anhydrous sodium sulphate and the solvent removed; yield 8·6 g. (68 per cent); b.p 152-5°/0·5 mm.; $[n]_D^{22}$, 1·5251. (Found: C, 74·90; H, 6·95; N, 5·49. $C_{16}H_{17}NO_2$ requires C, 75·29; H, 6·66; N, 5·49%.)

N-Phenyl-N(p-methoxybenzyl)-ethylamine — The amide (III) (0·5 g.) in ether (50 ml.) was added to a boiling solution of lithium aluminium hydride (1 g.) in ether (100 ml.) in a three-necked flask as before. The reaction mixture was then refluxed for 30 hr and worked up as in the case of *N*(*cis*-3-methoxyhexahydrobenzyl)-β-3,4-dimethoxyphenethylamine; yield 25 mg. (66 per cent); b.p. 135-7°/0·4 mm.; $[n]_D^{21}$, 1·5892. (Found: C, 78·56; H, 8·04; N, 5·87. $C_{16}H_{19}NO$ requires C, 79·61; H, 7·94; N, 5·80%.)

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HYDROLYSIS OF ETHYLENE DIBROMIDE BY MONOETHANOLAMINE AT ORDINARY TEMPERATURES

Monoethanolamine hydrolysis of ethylene dibromide in gaseous phase has been studied at different temperatures (21-38°C.). Ethylene dibromide undergoes complete hydrolysis within 3, 2, 1 and 0·5 hr at 21·1°, 25·0°, 31·0° and 37·8°C. respectively. The method is useful for the assay of ethylene dibromide from fumigation chambers.

Rauscher[1], while experimenting on the use of monoethanolamine for the hydrolysis of aliphatic and aromatic halogen compounds, reported complete hydrolysis of the halogen compounds in 49 cases. Hydrolysis of methyl bromide by monoethanolamine even at room temperature was employed by Stenger *et al.*[2] for the estimation of the fumigant. However, while subjecting ethylene dibromide to hydrolysis by monoethanolamine, at room temperatures low recoveries were obtained by Sinclair and Crandall[3]. They demonstrated complete dehydrobromination at 90°C. The resulting inorganic bromide was estimated by Volhard's method (Hillebrand and Lundell[4]).

With the increasing importance of ethylene dibromide as a tropical fumigant for grains, fruits and vegetables[5-7], the need for the development of simplified methods for routine assay of the fumigant has become imperative. In the present investigation the studies on the monoethanolamine dehydrobromination at various temperatures were carried out with a view to developing a simplified technique for the assay of ethylene dibromide during routine fumigation operations.

In preliminary experiments carried out in this laboratory on the assay of ethylene dibromide samples from a fumigation chamber, it was observed that it is possible to estimate the halogen compound almost quantitatively by carrying out hydrolysis of the fumigant by monoethanolamine even at room temperatures. A detailed study of hydrolysis of ethylene dibromide by monoethanolamine at various temperatures was, therefore, undertaken.

Thin-walled glass ampoules containing 1 ml. of monoethanolamine (E. Merck) were placed in iodine flasks (250 ml.) and ethylene dibromide (E. Merck) pipetted into it. The flasks were stoppered and weighed before and after adding the fumigant. Sufficient time was allowed for the fumigant to volatilize before releasing monoethanolamine by breaking the ampoules. The flasks were swirled vigorously to distribute monoethanolamine uniformly on the walls of the flasks. After the reaction period, inorganic bromide formed was estimated by Volhard's method and calculated for ethylene dibromide.

The iodine flasks containing the fumigant in the gaseous phase were subjected to hydrolysis at 21·1°, 25-29° (room temperature), 31·0° and 37·8°C. for 24 hr. The mean percentage of ethylene dibromide estimated as a result of hydrolysis at these temperatures was 103, 102, 102 and 104 respectively. The difference in the values obtained at any two temperatures was not significant. In a second experiment, the gas samples drawn from an all-glass fumigation chamber in 100 ml. sampling flasks were hydrolysed at room temperature for 24 hr and in another set at 90°C. for 30 min. in a hot-air oven[3]. The results obtained showed that the amount of ethylene dibromide accounted for in the sample was the same by both the methods. The difference in the values for ethylene dibromide in the samples by the two methods was 0·54 ± 0·46, which was not statistically significant (Table 1).

To determine the threshold time required for the complete hydrolysis of ethylene dibromide at various temperatures, ethylene dibromide in the gas phase and monoethanolamine were allowed to react at 21·1°, 25·0°, 31·0° and 37·8°C. for 30 min., 1, 2, 3, 4, 5, 6 and 8 hr. The results are graphically recorded in Fig. 1. The reaction was complete within 3, 2, 1 and 0·5 hr at 21·1°, 25·0°, 31·0° and 37·8°C. respectively. These results are contrary to the observations of Sinclair and Crandall[3].

The results of the present study indicate the possibility of utilizing monoethanolamine hydrolysis for determining ethylene dibromide concentrations in commercial fumatoria and warehouses during fumigation operations. The following procedure was followed. A two-way gas sampling flask (100 ml.) containing a 1-ml. ampoule of monoethanolamine was connected to the sampling line from a 200 cu. ft fumigation chamber and the gas was aspirated using a 1 litre capacity aspirator connected to the shorter limb of the sampling flask (Fig. 2). The aspirator was run till a certain representative sample of the gas had been drawn. Then the stopcocks on the sampling tube were closed and the flask shaken to break the monoethanolamine ampoule. The tube was kept in the open sun after distributing monoethanolamine well on to the walls of the tube. After 1 hr exposure to the sun, the resulting inorganic bromide was estimated by Volhard's method. The results show that the method is quite reliable and can be used for routine fumigant assay in godowns. By adopting this technique, evacuated flasks, vacuum pump, high temperatures and the necessary appliances required in the current method[3] were avoided, and the fumigant concentration could be determined in the warehouses during normal fumigation work. Due to the high price and non-availability of rapid gas analysers in India, such as the thermal conductivity units[8], this simplified technique of analysis at ordinary temperatures will prove advantageous for routine assay.

TABLE 1 — ESTIMATION OF ETHYLENE DIBROMIDE FROM FUMIGATION CHAMBER

Theoretical conc. *mg./litre*	Found *mg./litre*	Found %
Reaction at 23·9-25·0°C. for 24 hr		
96·00	90·69	94·47
82·18	59·98	72·99
78·73	66·12	83·98
79·69	64·63	81·10
112·00	69·42	61·98
	Mean	78·90
Reaction at 90°C. for 30 min.		
95·37	89·56	93·90
81·26	58·08	71·47
77·91	64·44	82·71
75·36	60·74	80·60
111·21	70·17	63·10
	Mean	78·36

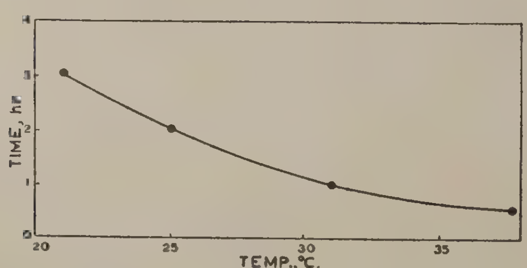

FIG. 1 — THRESHOLD TIME REQUIRED FOR COMPLETE HYDROLYSIS OF ETHYLENE DIBROMIDE AT DIFFERENT TEMPERATURES

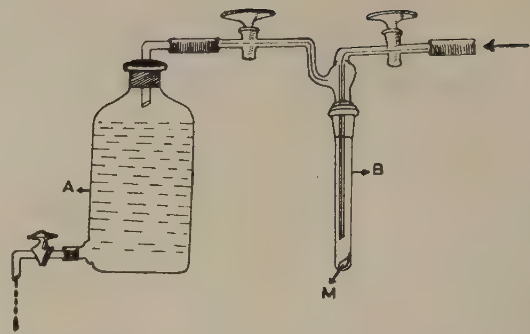

FIG. 2 — ASSEMBLY FOR GAS SAMPLING [A: aspirator; S: sampling and reaction tube; M: ampoule containing monoethanolamine. Arrow indicates connection to sampling line from fumigation chamber]

A portable fumigant analytical kit is being designed based on the above work, the constructional details and working of which will be described elsewhere.

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POLYESTERIFICATION OF POLYHYDROXY-POLYBASIC ACID: PART IV — POLYMOLECULARITY*

An attempt has been made to characterize the polymolecularity of three self-esters of the dibasic acid, 9,10-dihydroxyhexadecane-1,16-dicarboxylic acid, having low, medium and high degrees of polymerization by subjecting them to fractionation. The integral, differential weight and number distribution curves for the various fractions of the esters show that the fractions are distinct and that the ester molecules exhibit varying degrees of homogeneity in their chain length.

It was observed during the course of a study of the physico-chemical properties of 9,10-dihydroxyhexadecane-1,16-dicarboxylic acid that a polyester of the acid can be fractionated by adding a precipitant to a solution of the polyester[1]. The fractions separated have different molecular sizes and acid values and hence different solubilities. In the present study, an attempt has been made to characterize the polymolecularity of a few polyesters of 9,10-dihydroxyhexadecane-1,16-dicarboxylic acid consisting of molecules which are more or less alike in chemical composition and mode of linkages, but have heterogeneous molecular weights.

The method of precipitation followed was the same as developed by Mark[2] for cellulose and Kraemer and Lansing[3] and Signer and Gross[4] for polystyrenes. It consists in dissolving the entire sample in a chemically inert solvent and then precipitating the successive fractions by the addition of a suitable precipitant. Precipitation may also be accomplished by stepwise changes in temperature.

Three polyesters with different degrees of polymerization (DP), referred to as A, B and C, were prepared by isothermal heating of 9,10-dihydroxyhexadecane-1,16-dicarboxylic acid in a test tube as described previously[5] by suitable adjustment of temperature and duration of heating.

*The work reported in this paper forms part of the Ph.D. thesis submitted by the author and accepted by the Banaras Hindu University, 1959.

The fractionation of each polyester A, B and C was carried out as follows: An accurately weighed sample of the polyester was dissolved in dry acetone in a stoppered conical flask and placed in a thermostat ($30° \pm 1°$C.). Water from a burette was added to the solution and after the appearance of first turbidity (a black paper screen background was employed to detect the formation of precipitate), the precipitate was allowed to settle. The precipitate, after removing the supernatant liquid, was redissolved by warming and allowed to settle. It was separated by decantation, redissolved in acetone and the solvent evaporated for the recovery of the precipitate. To the decanted solution, a further amount of water was added from the burette and a second fraction was similarly collected and treated. The process of fractionation was continued till no further precipitate could be separated from the supernatant liquid. During precipitations, care was taken to see that the volume of the solutions did not increase excessively as the separation of the precipitate from large volumes of the solutions towards later stages always proved to be laborious and time-consuming.

The degree of polymerization (DP) of each of the fractions separating out was determined by the usual end-group method. The weight of each of the fractions separating out was determined and from it the cumulative percentage of the material remaining in the solutions at each stage was calculated. Since a small amount of the precipitate is always lost in the course of successive fractionations, it is not possible to recover the exact amount of the starting material from the solution and hence the cumulative percentages of the material remaining in solution have been calculated on the basis of the actual total amount recovered rather than on the amount of the starting material.

The degrees of polymerization of the various fractions have been plotted against the weights of polyesters remaining in solution at different stages. It is clear from the curves in Fig. 1 that the fractions obtained are sufficiently distinct, indicating efficient fractionation. The curve for polyester A shows an inflexion around DP 1·175 (Fig. 1). The steep portion near this point signifies a high concentration of molecules in this region (Fig. 2). Curve B has got two inflexion points near about DP 1·3 and 1·425. Curve C has also two points of inflexion at about DP 1·20 and 1·325. The maxima and minima corresponding to these inflexion points for different polyesters are evident from the differential weight distribution and differential number distribution curves presented in Figs. 2 and 3. In the integral weight distribution curves, all these points of inflexion are located before the point representing half-way in the

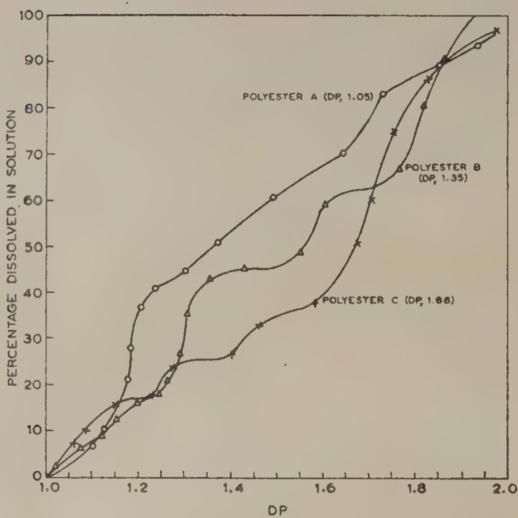

FIG. 1 — DEGREE OF POLYMERIZATION (DP) VERSUS WEIGHT OF POLYESTER REMAINING IN SOLUTION

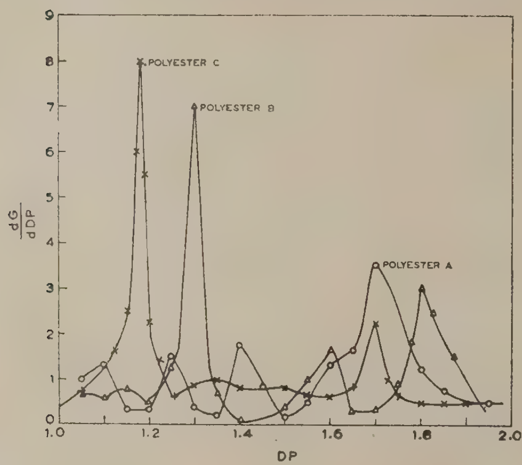

FIG. 2 — RATE OF GROWTH OF MOLECULES $\left(\frac{dG}{dDP}\right)$ VERSUS DEGREE OF POLYMERIZATION (DP)

range of total polymerization, indicating a more non-homogeneous character of the polymer molecule during this stage. In the latter half of the curve, the molecules conform to a more uniform composition.

In the differential weight distribution curves of the three polyesters (Fig. 2), the values represented along the ordinates have been obtained by graphic differentiation of the values presented in the integral weight distribution curves (Fig. 1) for each polymer. This is accomplished by dividing the change in the weight per cent (dG) by the change in the degree of polymerization (dDP). The curves for polyesters A and B are not so sharp and each has its own prominent peaks. The curve for polyester C is relatively smooth and sharp and the peaks are less prominent. Polyesters A and B have maxima and minima near about the region where the inflexion points are located, which probably means that these polymers are less homogeneous in chain length. But it has been found generally that such uniformity in chain length is always less pronounced in the case of synthetic polymers than for certain natural cellulose derivatives. In the differential number distribution curves shown in Fig. 3 the degree of polymerization has been plotted against the quotient of the functions of the weight distribution curves and the corresponding molecular weights or any values equivalent to the length of the polymer chain, e.g. the intrinsic viscosity of the function. The curves are almost similar to those in Fig. 2. Though homogeneity of chain length is an important physical property of polymers, an average value is usually taken to represent the different chain lengths and the non-homogeneity of chain length is ignored.

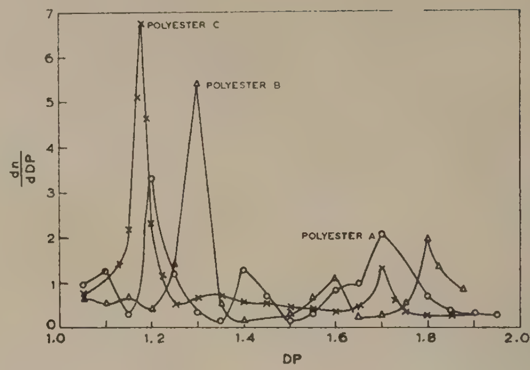

FIG. 3 — DIFFERENTIAL NUMBER DISTRIBUTION CURVES

The author is thankful to Dr S. V. Puntambekar, former Director, and Dr S. Krishnaswami, officiating Director, Indian Lac Research Institute, for their kind interest in the work and for helpful suggestions.

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Chemotherapeutic Studies on INH & Its Derivatives in Experimental Tuberculosis

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Four isonicotinic acid hydrazide derivatives have been tested for their antitubercular activities against *Mycobacterium tuberculosis* var. *hominis* $H_{37}R_v$ and its dihydrostreptomycin-resistant strain, both *in vitro* and *in vivo*. These compounds have bacteriostatic activity equivalent to that of INH.

RECENTLY Dhar[1] reported the preparation of four isonicotinic acid hydrazide (INH) derivatives which were designated as the symmetrical triazine obtained from INH and formaldehyde (P_1), crotonylidene isonicotinic acid hydrazone (P_3), the compound from mesityl oxide and INH (P_4) and cyclohexylidene isonicotinic acid hydrazone (P_8). This paper reports the results of chemotherapeutic studies on these derivatives *in vitro* and in experimental tuberculosis of guinea-pigs and mice.

Materials and methods

In vitro studies

The medium used for testing these derivatives was composed of: KH_2PO_4, 0·5; magnesium sulphate, 0·05; asparagin, 0·5; glycerol, 2·0; magnesium citrate, 0·15; sodium citrate, 0·15; ferric ammonium citrate, 0·002; malachite green, 0·0001; and agar (B.D.H.), 0·15 per cent.

The medium was mixed with INH in various concentrations and distributed in 4·5 ml. amounts in test tubes. Likewise P_1, P_3, P_4 and P_8 were mixed in various concentrations and distributed in test tubes in the same way. The test tubes containing the media were sterilized by autoclaving at 10 lb./sq. in. for 10 min. The medium containing drugs was inoculated with 0·1 ml. of a 10 days old culture of *Mycobacterium tuberculosis* var. *hominis*, strain $H_{37}R_v$, in Dubos liquid medium, and then incubated at 37°C. These tubes were examined after seven and fourteen days of incubation.

In vivo studies

Test I — Sixty normal male guinea-pigs weighing approximately 290 g. each and sixty Swiss albino mice weighing on the average 20-21 g. each were inoculated intracardially with 0·5 mg. (moist weight) and intravenously with 1·0 mg. (moist weight) respectively of 22 days old culture of *Mycobacterium tuberculosis* var. *hominis*, strain $H_{37}R_v$, on Lowenstein-Jensen medium, suspended in 0·2 ml. of $M/15$ disodium hydrogen phosphate buffer (*p*H 8·7). For the purpose of therapy both guinea-pigs and mice were divided into six groups of 10 each. One group of each species served as the untreated control. On the seventh day of infection, the other five groups were given INH and its derivatives. The drugs were administered orally once daily suspended in 4 per cent gum tragacanth at the dosage level of 17 mg./kg. for guinea-pigs and 50 mg./kg. for mice. The treatment was continued for fourteen days. Mortality was recorded daily. Criterion for detection of antituberculous activity was comparison of survival time between the control and the treated groups.

Test II — A second test was performed on guinea-pigs using dihydrostreptomycin-resistant *Mycobacterium tuberculosis* var. *hominis*, strain $H_{37}R_v$. In this test seventy-five tuberculin negative female

guinea-pigs, weighing about 370 g. each, were inoculated subcutaneously in the groin and over the sternum with 1·0 mg. (moist weight) of 26 days old culture of dihydrostreptomycin-resistant *Mycobacterium tuberculosis* var. *hominis*, strain $H_{37}R_v$, in the same manner described above. All animals reacted to old tuberculin on the nineteenth day of infection. Five animals were killed and autopsied on the twenty-fifth day of infection as the pretreatment controls. The remaining animals were divided into seven groups of 10 each and treated as follows.

One group received orally once daily, excepting Sundays, 1 ml. of 4 per cent gum tragacanth. Another group received subcutaneous injection of dihydrostreptomycin at the level of 10 mg./kg. dissolved in distilled water once daily excepting Sundays. Both these groups served as control. All other groups received by mouth once daily, excepting Sundays, the drugs under investigation suspended in 1·0 ml. of 4 per cent gum tragacanth at the level of 15 mg./kg. Treatment began on the twenty-fifth day of infection and lasted for 42 days. All surviving animals were killed 18 days after the treatment ended. At autopsy, the visible macroscopic lesions were recorded by giving the score 0 to 4 depending on the amount of gross tuberculous lesions in lung, liver, spleen and lymph gland. The moist weights of lung, liver and spleen were also recorded, as described by Gupta *et al.*[2].

Results

In vitro studies

The results of the experiment are given in Table 1. It was noted that after 14 days of incubation, 0·1 to 0·01 γ/ml. concentration of INH inhibited the growth of the organism while the control grew well after 5-7 days; P_1, P_3, P_4 and P_6, after 14 days of incubation, inhibited the growth of inoculum in the concentration between 1·0 to 0·01, 0·1 to 0·05, 0·1 to 0·01, and 1·0 γ/ml. respectively.

TABLE 1 — COMPARATIVE *IN VITRO* EFFECT OF INH AND P_1, P_3, P_4 AND P_6 ON TUBERCLE BACILLI

LAB. CODE No.	COMPOUND	MINIMAL INHIBITORY CONC. *γ/ml.*
INH	Isonicotinic acid hydrazide	0·1-0·01
P_1	Triazine from INH and formaldehyde	1·0-0·01
P_3	Crotonylidene isonicotinic acid hydrazone	0·1-0·05
P_4	Compound from mesityl oxide and INH	0·1-0·01
P_6	Cyclohexylidene isonicotinic acid hydrazone	1·0

In vivo studies

Test I — The experiment was terminated when 7 or more than 7 animals died due to specific cause from each group and the mean survival time was calculated from the data thus obtained by the method of best linear estimate given by Gupta[3]. The results are given in Table 2.

Test II — The distribution of animals according to necropsy score on different organs is recorded in Table 3. The final body weight and the moist weights of the lung, liver and spleen were statistically analysed (Table 4). On the basis of one character (organ weight) taken at a time, it was seen that groups treated with the derivative of INH differed from INH treated group. In such cases it became difficult to decide whether the overall effect was good or bad. In order to get an overall picture on the basis of all the three characters (lung weight, liver weight and spleen weight), Mahalanobis distance[4] (D^2 Statistics) between the two population (INH treated group and one of the derivatives treated group) was estimated and from that the significance between the INH treated group and one of the derivatives treated group was tested (Table 4).

TABLE 2 — COMPARATIVE ANTITUBERCULOUS ACTIVITY OF INH AND ITS DERIVATIVES

GROUP	GUINEA-PIGS Dosage: 17 *mg./kg./day*			MICE Dosage: 50 *mg./kg./day*		
	No. of animals dead*	Calc. mean survival time in log of No. of days	Standard deviation	No. of animals dead	Calc. mean survival time in log of No. of days	Standard deviation
Control	10	1·4483	0·1333	10	1·3057	0·0590
INH	8	2·0482	0·2698	9	1·8452	0·1954
P_1	7	2·1649	0·2408	9	1·8367	0·1876
P_3	9	2·0440	0·1322	7	1·8034	0·1991
P_4	9	2·0419	0·1384	10	1·8413	0·1082
P_6	9	2·0379	0·1062	10	1·8553	0·0493

*Animals showing macroscopic evidence of tuberculosis at necropsy.

TABLE 3 — DISTRIBUTION OF ANIMALS ACCORDING TO NECROPSY SCORES ON DIFFERENT ORGANS

Group	No. of animals considered	Lung score					Liver score					Spleen score					Lymph gland score				
		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Untreated control*	9	1	3	1	3	1	0	4	1	0	4	3	2	0	1	3	0	0	0	4	5
Streptomycin treated	10	0	4	2	2	2	1	0	2	2	5	0	2	1	1	6	0	0	0	0	10
INH treated	10	6	3	1	0	0	10	0	0	0	0	8	2	0	0	0	0	0	0	10	0
P_1 treated	10	5	5	0	0	0	10	0	0	0	0	8	2	0	0	0	0	0	0	9	1
P_3 treated	10	3	5	1	1	0	10	0	0	0	0	10	0	0	0	0	0	0	0	9	1
P_4 treated	10	2	7	1	0	0	9	0	0	1	0	7	3	0	0	0	0	0	0	8	2
P_6 treated	10	1	6	3	0	0	10	0	0	0	0	10	0	0	0	0	0	0	0	10	0

*One animal died of non-specific cause.

TABLE 4 — STATISTICAL ANALYSIS OF BODY AND ORGAN WEIGHTS

Group	Body wt	Lung wt	Liver wt	Spleen wt	Comparison	Probability
Untreated control	425·56±50·020	5·74±0·372	20·26±0·988	4·62±1·505	—	—
Streptomycin treated	435·00±35·010	4·82±0·353	23·14±2·900	9·39±1·961	—	—
INH treated	537·00±16·740	3·89±0·138	13·47±0·652	0·74±0·039	—	—
P_1 treated	473·40±19·110	4·18±0·135	13·85±0·538	0·84±0·057	INH vs P_1	$P>0·20$
P_3 treated	477·00±27·510	4·07±0·198	12·27±0·672	0·73±0·048	INH vs P_3	$P>0·20$
P_4 treated	456·00±22·560	4·08±0·219	11·94±0·506	0·88±0·091	INH vs P_4	$0·10<P<0·20$
P_6 treated	445·30±12·790	3·93±0·202	11·54±0·484	0·65±0·028	INH vs P_6	$P<0·01$
Pretreatment control	379·80± 4·044	4·45±0·511	11·53±0·575	1·08±0·213	—	—

Discussion

The results of *in vitro* studies (Table 1) show that minimal inhibitory concentrations of INH and its derivatives are more or less the same, since by prolonging the period of incubation it has been observed that the end point shifts. This phenomenon of shift in the end point confirms the earlier reports on INH already published by Knox *et al.*[5].

From the data presented in Table 2 it can be observed that there is an appreciable difference between untreated and treated groups both in guinea-pigs and mice whereas among the treated groups there appears to be no statistically significant difference.

On analysis of necropsy score (Table 3) it is seen that the distribution of animals in streptomycin treated group is similar to that of animals in untreated control group, thus proving that the culture used to infect the guinea-pigs was genuinely of streptomycin-resistant strain. Furthermore, the distribution of INH treated animals is similar to that of all the derivatives treated group with respect to liver, spleen and gland involvement. In case of lung involvement, P_1 treated group is of the same order as INH treated group. In the remaining groups, the lung involvement is slightly more than in INH treated group though not significant. From the data so far obtained it may be concluded that all the derivatives are more or less equally active as INH. But on analysis of the data in Table 4 it will be seen that P_1, P_3 and P_4 are as good as INH, whereas P_6 is significantly better than INH in the sense that the organ weights of P_6 treated group are in general lesser than those in INH treated group. It has been reported in a British Patent[6] that tuberculostatic activity of P_1 in animals is superior to INH, but there is no mention of the kind of animal and the strain of culture used for the experiment. It was reported by Bernstein *et al.*[7] that P_6 is quite active against *Mycobacterium tuberculosis* var. *bovis*, strain Ravenel, in mice.

Acknowledgement

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Pectic Enzymes Secreted by Fungi & Their Action on Jute Bark

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A large number of species of fungi have been grown on a pectin medium and the filtrates tested for polygalacturonase activity, as shown by lowering of viscosity of apple pectin solution. No relation between mycelial growth and enzyme production has been observed, and often, in spite of heavy growth, there is little adaptive enzyme formation. *Penicillium* species have been found to secrete the most potent pectic enzymes. Of the five nitrogen sources tried, ammonium sulphate and nitrate are most favourable for enzyme formation, and sodium nitrate and peptone the least. Pectin isolated from jute cuttings has been found to behave somewhat differently from, and to be more resistant than, apple pectin as a substrate. Polygalacturonase, pectinesterase and protopectinase activities have been found to differ sometimes widely in the same filtrate. While the protopectinase enzyme seems to be the most potent in the softening of green jute bark, activity on dry cuttings could not be correlated with any of the enzymes. The evidence presented supports previous indications that the pectinous material in jute bark undergoes changes on drying.

A CONSIDERABLE amount of information has been gathered on pectic enzymes secreted by fungi and bacteria. Several classes of these enzymes have been distinguished, since for one thing pectic materials are not uniform in composition and, secondly, in the plant tissue they often exist in combination with other cell constituents. The essential and principal component of all pectic substances is D-galacturonic acid; as generally defined, pectic acid is a polymer of this unit in the pyranose form linked in α-configuration in the 1-4 positions. The carboxyl groups are often combined in the methyl ester form; the general name pectin is used to designate a compound with a relatively high ester content, while pectinic acid is the term used for polygalacturonic acid containing more than a negligible proportion of methyl ester groups. Protopectin is a rather ill-defined water-insoluble parent pectic substance of plants, which upon restricted hydrolysis yields pectinic acids. Carboxyl groups are sometimes combined with various cations to form salts.

Pectic enzymes are of the following three main types: (a) protopectinase, which attacks protopectin, apparently through hydrolysis, and renders the pectinous matter soluble; (b) polygalacturonase (PG), which catalyses the hydrolytic splitting of the glycosidic linkage between galacturonic acid residues, producing the latter as the final product; the name depolymerase has been given to an enzyme which similarly reduces molecular size but without eventual hydrolysis to the monomer[1]; and (c) pectinesterase (PE), which is responsible for the hydrolysis of methyl ester groups, producing free carboxyl groups.

In this study, a survey has been made of the production of pectic enzymes by fungi, isolated from various sources and mostly belonging to the Imperfect class, with emphasis on the optimum conditions for the production of PG. The ultimate object was the recovery of fibre from under-retted jute bark corresponding to the bottom portion of the plant. Very little is known of the nature of jute pectin binding the fibres together; a low methoxyl content (4 per cent) was reported by Bose[2] for 'pectin' prepared from mechanically separated jute fibres, and galacturonic acid was identified by chromatography as the main product of hydrolysis by fungal PG.

Materials and methods

'Elpex' apple pectin (Grade 40X, William Evans & Co. Ltd, Hereford) was used, both in the medium for growing fungi and as substrate for the action of PG and PE. The composition of the basic growth

medium was: pectin, 10 g.; NH_4NO_3, 1 g.; K_2HPO_4, 0·75 g.; KH_2PO_4, 0·25 g.; $MgSO_4.7H_2O$, 0·5 g.; KCl, 0·5 g.; and distilled water, 1000 ml.; the *p*H of the medium was 5·1. The *p*H and nitrogen source were, however, varied in the later experiments.

The medium was taken in 30 ml. quantities in 125 ml. pyrex conical flasks and sterilized at 10 lb./sq. in. for 20 min. The flasks, after inoculation, were incubated at 30°C. for 9 days. The growth medium was then filtered through fine muslin and the filtrate tested for exocellular pectic enzyme activity.

The fungi used were taken from the culture collection maintained in this laboratory, each with a separate reference number. Many of them were cellulose decomposers. The stock cultures were grown alternately on potato-dextrose agar and malt agar, and preserved in a refrigerator.

The fall in viscosity of apple pectin solution was determined as follows. Ten ml. of a 1·5 per cent solution of apple pectin in 0·05*M* phthalate buffer at *p*H 4·0 were mixed with 1 ml. of the filtrate to be tested. Enzyme action was allowed to proceed at 37°C. for exactly 30 min. The time of flow of the hydrolysate was then determined in an Ostwald viscometer at 25°C. Distilled water as well as pectin solution incubated with boiled enzyme was used as control. Strictly speaking, this method probably measures the combined action of PG, depolymerase and PE, since the true substrate of PG is said to be pectic acid[3]; the term PEGS has, therefore, been used to designate this complex activity.

The method of preparation of jute 'pectin', obtained from the under-retted portions of jute bark known as cuttings, was as follows: the cuttings were powdered in a Wiley mill and extracted with 10 vol. of 0·5 per cent ammonium oxalate solution for 24 hr at 85°C. The extract was filtered, the residue being washed with ammonium oxalate solution. The filtrate was concentrated to less than a fourth of the initial volume and the pectin then precipitated by adding 3·5 vol. of 95 per cent ethanol acidified with hydrochloric acid. The crude product thus obtained was washed with acidified ethanol until free from oxalate. Further purification was done by repeatedly dissolving it in boiling water and precipitating with acidified ethanol, finally washing free of hydrochloric acid with 95 per cent ethanol. The purified pectin was then dried in vacuum over calcium chloride. For test purposes, a 3 per cent solution of this pectin was used in order to obtain a proper consistency. Enzyme solutions were prepared by growing the fungi on media containing the most suitable nitrogen source. The rest of the procedure was exactly as in the previous test.

Reducing power was measured by the Jansen and McDonell modification[4] of the Willstätter-Schudel hypoiodide method. One ml. of enzyme solution was added to 10 ml. of 1·5 per cent apple pectin solution in 0·05*M* phthalate buffer at *p*H 4·0 and the flasks incubated at 37°C. At intervals of 0, 30 and 60 min., 5 ml. aliquots were withdrawn and allowed to react with standard iodine solution as specified, the unreacted iodine being then titrated with *N*/50 thiosulphate solution. Titres for 30 min. and 60 min. were subtracted from that for 0 min. to arrive at a figure corresponding to the reducing power of the pectin hydrolysate. This was corrected by subtracting the corresponding figure for boiled enzyme, which, however, was usually zero. Experiments were always carried out in duplicate and the mean reading calculated.

A qualitative estimate of the PE activity was made on the basis of the carboxyl groups produced as a result of de-esterification of the pectin molecule. A 1 per cent apple pectin solution was adjusted to *p*H 6·5 with 0·1*N* NaOH solution using methyl red as indicator. To 40 ml. of this solution were added 2 ml. of methyl red and 5 ml. of fungal filtrate. Incubation was at 30°C. for 18·5 hr. The change of colour of the indicator towards pink at the end of this period was taken as the index of de-esterification.

Protopectinase activity was determined by the simple method described by Brown[5]. Two 1·2 cm. square potato slices of thickness 0·5 mm. were placed in 5 ml. of enzyme solution contained in narrow weighing bottles and these incubated at 37°C. At intervals of 15 min. an estimate of the degree of maceration was made by pulling the strips in opposite directions by means of blunt forceps. The results were recorded with a scale of signs ranging from — indicating no softening, to +++ indicating no resistance to pull.

The action of some enzyme solutions was tested on green jute bark and dry jute cuttings. For this purpose, the filtrates were taken in large test tubes, each containing a 2 × 1·5 cm. strip of bark or cutting. After the addition of two drops of toluene, the tubes were stoppered and incubated at 37°C. for 20-22 hr. Testing was done by pulling the strips laterally and the degree of softening represented as before, the maximum indicating that the fibres could be separated by gentle pulling.

Results and discussion

In a preliminary experiment 92 fungi were tested for PEGS activity. Flow time for water was found to be 16·7 sec. and that for substrate plus boiled enzyme 47·5 sec. A flow time twice that of water was arbitrarily chosen for separating the promising

fungi for further experiments. Only 29 organisms (26 species) fell within this category, and some among them reduced the flow time to nearly that of water. No relation was observed between mycelial growth and PEGS activity and most of the discarded organisms grew well on the pectin medium, although a few gave poor or no growth. The results with 25 of the 29 organisms are given in Table 1, the other four being *Aspergillus terreus* 6, *A. ustus* 29, *Penicillium versicolor* type 30 and *P. fellutanum* 125.

The fungi which produced appreciable PEGS all belonged to the genera *Aspergillus* and *Penicillium*, excepting only two (Table 1). Those tried but found to produce too little enzyme were: *Aspergillus chevalieri* 88, *A. flavipes* 5, *A. flavus* 2, *A. nidulans* 12, *A. oryzae* type 59, *A. sydowi* 44, *A. unguis* 143, *Penicillium adametzi* 128, *P. canescens* series 136, *P. citrinum* 37, *P. janthinellum* series 153, *P. notatum* 138, *P. roseopurpureum* 148, *P. steckii* 137, *P. tardum* 132, *P. variabile* 34, *P. vermiculatum* 9, *P.* spp. 84, 91 and 135, *Chaetomium aureum* 145, *C. brasiliense* 117, *C. cancroideum* 108, *C. cochliodes* 110, *C. dolichotrichum* 111, *C. elatum* 116, *C. funicola* 112, *C. globosum* 79·1, *C. indicum* 75, *C. microcephalum* 113, *C. ochraceum* 109, *C. pachypodioides* 114, *C. trigonosporum* 151, *C.* sp. 115, *Acontinum* sp. 156, *Actinomyces* sp. 142, *Alternaria* sp. 60, *Cephalosporium* spp. 131, 147 and 159, *Cladosporium* sp. 32, *Curvularia lunata* 10, *Cunninghamella* sp. 80, *Fusarium* sp. 149, *Gliomastix convoluta* 152, *Helminthosporium* spp. 17 and 144, *Heterosporium* sp. 157, *Memnoniella echinata* 73, *M. echinata* 133, *Metarrhizium anisopliae* 106, *Monilia sitophila* 156, *Myrothecium verrucaria* 139, *Paecilomyces varioti* 18, *Pullularia pullulans* 154, *P. pullulans* 160, *Scopulariopsis* sp. 120, *Stachybotrys atra* 70, *Stemphylium* sp. 107, *Stysanus* sp. 141, *Syncephalastrum* sp. 19 and unidentified fungi 35 and 119. Of these, *Chaetomium elatum*, *C. microcephalum*, *C. padypodioides* and *C. trigonosporium* gave only slight growth on the pectin medium, and *Actinomyces* sp. 142, *Memnoniella echinata* 133 and unidentified species 35 and 119 did not grow at all.

Since enzymes are mainly proteins in composition and the form of the source of nitrogen supplied to the fungus may have a bearing on their synthesis, four other nitrogen sources, namely ammonium sulphate, sodium nitrate, peptone (Difco) and asparagine (Merck), were also tested. Nitrogen content was kept in each case at the same level as in the ammonium nitrate medium, i.e. 0·035 per cent. Enzyme extracts were prepared and tested as before after 9 days' growth. The mycelia were harvested at the same time and their dry weight determined. Changes in *p*H due to growth were also recorded. Data concerning the two most favourable nitrogen sources for enzyme production and the most favourable nitrogen source for mycelial development are given in Table 1.

Ammonium nitrogen was most often best suited for enzyme production, 15 fungi giving maximum yield with the nitrate and 6 with the sulphate of ammonium. As expected, considerable acid was produced in the ammonium salts media due to the using up of the basic radicle. On the other hand, the *p*H generally rose with the other nitrogen sources. However, it does not seem that a low *p*H in itself favours enzyme production, since the latter was maximum with asparagine in seven instances. The corresponding figures for sodium nitrate and for peptone were one and nil respectively. The poor result with peptone was certainly not due to the inability of the fungi to utilize it, since in fact peptone most often gave the maximum growth, followed closely by asparagine. Once again, little correlation is seen between vegetative growth and enzyme production.

The action of these enzymes was then tested on jute pectin. Several filtrates showed rather poor PEGS activity on jute pectin, in striking contrast to their action on apple pectin. Fungi whose filtrates reduced the flow time to less than 21 sec. were selected for further studies on increase in reducing value, PE and protopectinase activity. These were 11 in number, 9 being *Penicillium* species. Of these, only 6 were among the first 11 organisms giving the most potent enzymes as regards apple pectin. Whereas enzymes from *A. japonicus* and *Heterosporium* sp. were more potent on jute pectin compared to apple pectin, those from *P. oxalicum* and *P. variabile* were relatively less potent, while *P. implicatum* was nearly the most effective fungus for the lowering of viscosity of both pectins. These results suggest structural differences between the two pectins.

The increase in reducing power following the action of PEGS on pectin and breakdown of the polygalacturonic acid chain is due to an increase in aldehydic end-groups. For measurements of reducing power, all the five nitrogen sources were used in the medium to grow the fungi, so that comparisons could be made with changes in flow time (Table 1). Titres for 30 min. were usually almost exactly half of those for 60 min., and only the latter are set out in Table 2, along with the flow-time values of jute pectin solution.

It is seen from the results that generally the most suitable nitrogen source was the same as that obtained in the previous experiment with apple pectin where fall in viscosity was the criterion. A rather marked discrepancy is, however, seen with *P. brefeldianum*, the best nitrogen sources being ammonium nitrate and asparagine in this and the previous experiment respectively. Ammonium salts, followed by asparagine,

TABLE 1 — EFFECT OF VARIOUS NITROGEN SOURCES ON THE PRODUCTION OF PEGS*

N source	Flow time sec.	Final *p*H	Mycelial wt mg.
***Aspergillus atropurpureus* 92**			
N_1	20·2	2·7	84
N_2	20·2	2·8	82
N_4	43·0	6·4	102
***A. fumigatus* 14**			
N_3	27·5	7·0	60
N_5	24·0	7·3	93
***A. japonicus* 140**			
N_2	24·7	3·0	93
N_4	27·3	5·7	114
N_5	23·3	5·7	109
***A. niger* 3**			
N_1	19·0	2·5	74
N_2	18·5	3·5	82
N_4	20·5	6·0	107
***A. proliferans* 23**			
N_1	27·0	2·6	72
N_2	28·0	3·4	74
N_5	34·4	8·0	108
***Penicillium brefeldianum* 146**			
N_3	20·5	8·1	76
N_4	21·4	6·9	120
N_5	19·2	8·2	93
***P. cyaneum* 124**			
N_1	20·6	2·8	91
N_2	19·5	4·2	84
N_4	34·0	7·3	131
***P. fellutanum* 7**			
N_1	19·8	2·6	80
N_2	19·8	2·8	80
N_4	26·0	6·5	125
***P. funiculosum* series 150**			
N_1	25·8	2·8	66
N_2	25·5	3·3	65
N_4	44·3	6·7	104
***P. implicatum* 53**			
N_3	21·8	6·8	83
N_4	24·5	6·8	109
N_5	20·7	6·8	93
***P. implicatum* series 101**			
N_1	18·5	2·8	68
N_2	19·3	6·4	73
N_4	29·5	7·8	122
***P. luteum* 28**			
N_1	20·3	2·8	65
N_2	20·0	2·8	64
N_5	29·7	6·9	92
***P. oxalicum* 96**			
N_2	20·7	2·8	60
N_4	27·7	5·8	93
N_5	18·3	5·3	75
***P. purpurogenum* 134**			
N_1	20·3	2·8	65
N_2	19·7	2·8	66
N_4	42·0	5·8	92
***P. rubrum* 127**			
N_4	21·3	5·5	91
N_5	21·0	5·7	97
***P. simplicissimum* 4**			
N_2	23·0	2·8	89
N_4	32·0	6·9	137
N_5	24·5	8·0	86
***P. variabile* 121**			
N_1	20·3	2·8	61
N_2	19·0	4·2	70
N_4	30·0	6·7	124
***P. vermiculatum* 77C**			
N_1	22·6	2·8	57
N_2	22·3	3·0	65
N_4	38·8	7·0	101
***P. verruculosum* 158**			
N_1	24·5	2·8	70
N_2	24·2	2·9	74
N_4	30·7	5·7	102
***P. wortmanni* 126·24**			
N_2	23·5	2·8	91
N_3	22·7	5·6	87
N_4	25·5	5·7	128
***P. wortmanni* 130·30**			
N_1	20·0	2·8	80
N_2	20·3	2·8	92
N_4	24·0	5·7	127
***P.* sp. 40**			
N_1	20·5	3·0	61
N_2	23·5	6·2	60
N_4	41·3	7·3	102
***P.* sp. 98**			
N_1	18·8	3·0	44
N_2	18·2	4·5	57
N_4	39·6	7·5	99
***Heterosporium* sp. 104**			
N_1	28·4	2·8	83
N_2	28·8	3·0	86
N_4	43·5	8·0	124
***Trichoderma viride* 24**			
N_1	29·7	2·7	50
N_2	30·5	2·6	53
N_4	46·0	6·6	67

[$N_1 = (NH_4)_2SO_4$; $N_2 = NH_4NO_3$; $N_3 = NaNO_3$; N_4 = peptone; N_5 = asparagine; initial *p*H values of media: N_1, N_2 and N_3 = 5·1; N_4 = 5·4; N_5 = 4·7; flow time for distilled water = 16·7 sec., for substrate and boiled enzyme = 47·5 sec.]

*Extent of PEGS production is expressed in terms of fall in viscosity of pectin solution.

TABLE 2 — ACTION OF PEGS IN LOWERING THE VISCOSITY OF JUTE PECTIN AND INCREASING THE REDUCING POWER OF APPLE PECTIN

Fungus and Ref. No.	N source	Jute pectin, flow time sec.	Apple pectin, reducing power ml.				
			$(NH_4)_2SO_4$	NH_4NO_3	$NaNO_3$	Peptone	Asparagine
Aspergillus atropurpureus 92	N_2	22·0	—	—	—	—	—
A. fumigatus 14	N_5	23·8	—	—	—	—	—
A. japonicus 140	N_5	20·2	0·60	1·20	1·20	1·15	2·00
A. niger 3	N_2	22·5	—	—	—	—	—
A. proliferans 23	N_1	21·0	—	—	—	—	—
Penicillium brefeldianum 146	N_5	20·2	0·30	1·55	0·95	0·80	0·95
P. cyaneum 124	N_2	21·8	—	—	—	—	—
P. fellutanum 7	N_2	22·5	—	—	—	—	—
P. funiculosum series 150	N_2	20·2	1·40	0·90	0·35	0·45	0·65
P. implicatum 53	N_5	21·8	—	—	—	—	—
P. implicatum series 101	N_1	18·8	2·10	1·45	0·50	0·35	0·50
P. luteum 28	N_2	20·8	1·00	1·05	0·90	0·65	1·20
P. oxalicum 96	N_5	20·2	0·95	0·80	0·55	0·20	1·00
P. purpurogenum 134	N_2	21·8	—	—	—	—	—
P. rubrum 127	N_5	21·7	—	—	—	—	—
P. simplicissimum 4	N_2	23·5	—	—	—	—	—
P. variabile 121	N_2	20·5	1·00	1·15	0·70	0·45	0·65
P. vermiculatum 77C	N_2	20·7	0·90	0·65	0·15	0·15	0·05
P. verruculosum 158	N_2	21·5	—	—	—	—	—
P. wortmanni 126·24	N_3	23·5	—	—	—	—	—
P. wortmanni 130·30	N_1	20·2	0·95	0·90	0·10	0·20	0·40
P. sp. 40	N_1	20·2	1·55	0·75	0·50	0·30	0·25
P. sp. 98	N_2	22·0	—	—	—	—	—
Heterosporium sp. 104	N_1	18·9	0·20	0·20	0·15	0·10	0·15
Trichoderma viride 24	N_1	21·0	—	—	—	—	—
Control (boiled enzyme)	—	24·3	—	—	—	—	—

[N_1 = $(NH_4)_2SO_4$; N_2 = NH_4NO_3; N_3 = $NaNO_3$; N_4 = peptone; N_5 = asparagine]

again gave the most potent enzymes. Among the fungi tested, *A. japonicus*, *P. brefeldianum*, *P. implicatum* and *P.* sp. 40 were the most active in enzyme production. Of these, only *P. implicatum* and *P. brefeldianum* occupy the top positions when these 11 fungi are arranged in order of reduced flow time (Table 1), enzymes from *P. oxalicum*, *P. variabile* and *P. wortmanni* being relatively more effective in lowering viscosity. The enzyme from the *Heterosporium* species was the least active in both cases. It is of course well known that fall in viscosity and increase in reducing value do not always run in parallel. Kertesz[6] has suggested the association of chains into larger units by linkages more vulnerable than the 1-4 glycosidic bond; the breakdown of these secondary bonds would only lower the viscosity. On the other hand, end-wise splitting of the polygalacturonic chain would increase the number of reducing groups without much affecting the viscosity. Such results are all the more to be expected when the enzyme source is mixed, as in the present case.

A qualitative assessment of the PE activity of these fungi was made with enzyme solutions prepared from media containing the most suitable nitrogen source as obtained from Table 1. With boiled enzymes there was no colour change towards pink, and so also with unboiled enzymes from *P. luteum*, *P. vermiculatum* and *Heterosporium* sp. A slight colour change was obtained from the filtrate of *P. brefeldianum*, and the remaining seven fungi gave filtrates showing marked activity, as indicated by a deep pink colour. Among these latter were species with high as well as low PEGS activity, such as *P. implicatum* and *P. vermiculatum* respectively.

For the study of protopectinase activity the fungi were grown on media containing the optimum nitrogen sources as obtained from Table 1. The filtrates were adjusted to a wide range of pH values (3·0-8·0) by means of 0·1N HCl or 0·1N NaOH. There was no maceration with boiled enzyme even after 90 min. The results were reproducible (Table 3).

It is seen from the results given in Table 3 that many of the filtrates were very active in protopectinase, complete softening being obtained in the minimum time in four cases. Of these latter, three had also proved to be very active in PEGS enzymes for reducing viscosity of apple pectin, but the fourth fungus, *Heterosporium* sp., had been poor in this respect, although its filtrate had been very active on jute pectin. From an analysis of all the filtrates, it appears that protopectinase activity, as determined here, was not identical with any of the other enzymes tested. Maximum protopectinase activity persisted in all cases even at the lowest pH tested (3·0), the optimum range being usually 3·0-5·0, except with the enzyme from *P. brefeldianum* which was equally active over the whole range. Previous workers[7] have also found the optimum pH range to be 3·0-5·0.

TABLE 3 — EFFECT OF *p*H ON PROTOPECTINASE ACTIVITY

N source	*p*H	Activity 15 min.	30 min.	45 min.	1½ hr.
***Aspergillus japonicus* 140**					
N_5	3·0	−	++	+++	+++
	4·0	−	++	+++	+++
	5·0	−	+	+++	+++
	6·0	−	−	−	−
***Penicillium brefeldianum* 146**					
N_5	3·0	+++	+++	+++	+++
	4·0	+++	+++	+++	+++
	5·0	+++	+++	+++	+++
	6·0	+++	+++	+++	+++
	7·0	+++	+++	+++	+++
	8·0	+++	+++	+++	+++
***P. funiculosum* 150**					
N_2	3·0	+	+++	+++	+++
	4·0	+	+++	+++	+++
	5·0	+	++	+++	+++
	6·0	−	−	−	−
***P. implicatum* series 101**					
N_1	3·0	+++	+++	+++	+++
	4·0	++	+++	+++	+++
	5·0	++	+++	+++	+++
	6·0	−	+	++	+++
	7·0	−	−	−	+
	8·0	−	−	−	−
***P. luteum* 28**					
N_2	3·0	++	+++	+++	+++
	4·0	−	++	+++	+++
	5·0	−	−	+	+++
	6·0	−	−	−	−
***P. oxalicum* 96**					
N_5	3·0	+++	+++	+++	+++
	4·0	+++	+++	+++	+++
	5·0	++	+++	+++	+++
	6·0	−	++	+++	+++
	7·0	−	+	++	+++
	8·0	−	−	++	+++
***P. variabile* 121**					
N_2	3·0	++	+++	+++	+++
	4·0	+	+++	+++	+++
	5·0	+	+++	+++	+++
	6·0	−	−	−	−
***P. vermiculatum* 77C**					
N_2	3·0	++	+++	+++	+++
	4·0	++	+++	+++	+++
	5·0	++	+++	+++	+++
	6·0	−	−	−	−
***P. wortmanni* 130·30**					
N_1	3·0	−	++	+++	+++
	4·0	−	+	++	+++
	5·0	−	−	+	+++
	6·0	−	−	−	−
***P.* sp. 40**					
N_1	3·0	−	+++	+++	+++
	4·0	−	+	+++	+++
	5·0	−	+	++	+++
	6·0	−	−	+	++
	7·0	−	−	−	−
***Heterosporium* sp. 104**					
N_1	3·0	+++	+++	+++	+++
	4·0	++	+++	+++	+++
	5·0	++	+++	+++	+++
	6·0	−	−	+	+++
	7·0	−	−	−	−

[$N_1 = (NH_4)_2SO_4$; $N_2 = NH_4NO_3$; N_5 = asparagine; −, no softening; +++, complete maceration]

TABLE 4 — SOFTENING OF GREEN JUTE BARK AND CUTTINGS BY ENZYMES

Fungus and Ref. No.	N source	Green bark	Cutting
Aspergillus japonicus 140	Asparagine	+++	++
Penicillium brefeldianum 146	do	+++	++
P. funiculosum 150	NH_4NO_3	+	−
P. implicatum series 101	$(NH_4)_2SO_4$	+++	+
P. luteum 28	NH_4NO_3	+	−
P. oxalicum 96	Asparagine	+++	−
P. variabile 121	NH_4NO_3	++	+
P. vermiculatum 77C	do	++	−
P. wortmanni 130·30	$(NH_4)_2SO_4$	+	−
P. sp. 40	do	+++	−
Heterosporium sp. 104	do	+++	+

[−, no softening; +++, maximum softening]

The action of these enzymes was also tested on green jute bark and dry jute cuttings, the filtrates being adjusted to *p*H 3·0. No softening was observed with boiled enzyme. The results given in Table 4 indicate that all the four filtrates which showed the maximum protopectinase activity with potato slices also gave maximum activity with green jute bark; in addition, enzymes from *A. japonicus* and *P.* sp. 40 gave equally high activity. Dry cuttings were clearly more resistant than green bark, satisfactory fibre separation never being obtained. The relative potencies of the filtrates could not be clearly correlated with their protopectinase and other enzyme activities as determined earlier, including the effect on 'pectin' isolated from cuttings. This again confirms previous indications[8] that the pectinous binding material of jute stem undergoes chemical and/or physical changes on drying which makes it more resistant to biological attack. There is also some indication that the chemically separated 'pectin' from cuttings is not identical with this native resistant product.

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Studies on *Idli* Fermentation: Part I — Some Accompanying Changes in the Batter

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An increase in non-protein nitrogen and a decrease in reducing sugars have been observed during fermentation of *Idli* (a popular breakfast dish in South India) batters; the batters are usually prepared by soaking rice (*Oryza sativum*) and decuticled black gram (*Phaseolus mungo*) dhal in water, grinding them separately, mixing, and allowing the mixture to ferment overnight. Both titratable acidity and the volume of the batter increase as a result of fermentation and have been used as criteria for judging the progress of fermentation. A temperature range of 25-30°C. has been found to be optimal for the fermentation. Temperatures up to 40°C. accelerated the rate, but some undesirable smell occasionally developed at higher temperatures.

Presoaking of black gram dhal prior to grinding in the traditional methods has been established to be an important step in the fermentation. The possibilities of a 'Flour Presoaking Method' and a 'Composite Dry Mix Method' for *Idli* making to eliminate the need for wet grinding of black gram dhal and rice are indicated by the data.

That both yeasts and bacteria participate in the fermentation has been shown using penicillin G and chlortetracyclin as selective inhibitors. Acid and gas production have been found to be mostly dependent on the growth of microbes belonging to the bacterial group.

FERMENTATION of natural foods as a method of leavening has been known from the earliest times, the best known example being yeast fermentation of wheat dough for baking of bread. A similar product which is leavened by natural fermentation and which is a popular breakfast dish over most parts of India, especially in the South, is the *Idli*. It is soft, spongy, tasty and easily digestible. The basic ingredients of this preparation are rice (*Oryza sativum*) and decuticled black gram (*Phaseolus mungo*) dhal. The two ingredients are soaked in water and ground separately in a stone mortar and pestle, mixed, and the batter allowed to ferment overnight. Lewis and Johar[1,2] studied the nature of microorganisms in fermenting *Idli* batters. The work reported here relates to methods for fixing criteria for optimum 'ripeness' of the batter, effect of temperature on fermentation, the relative participation of different microorganisms in producing acid and gas and comparison of different methods of fermentation, with respect to both physical and chemical changes in the batter and *Idli* quality. Practical applications of these data are described elsewhere.

Materials and methods

Traditional method (control) — Decuticled black gram dhal was soaked in water for 6 hr and then ground to a fine paste and mixed with 2 parts by weight of parboiled rice semolina (corresponding to −18+20 B.S.I. mesh), salt 2·8 per cent and water 2·2 parts on the weight of dry solids, and allowed to ferment for another 14-16 hr.

Dry mix composition — One part of finely ground black gram flour (−52+56 B.S.I. mesh) and 2 parts of rice semolina (−18+20 B.S.I. mesh) and containing 2·8 per cent sodium chloride were mixed together and stored. As and when required, the batter was prepared from this stock by uniformly dispersing it in 2·2 parts water into a homogeneous consistency.

Dry mix composition with yeast — The mix was prepared as in dry mix composition except that it contained 20 mg. of dry baker's yeast (D.C.L.); a small amount of lactic curds inoculum (1·0 ml. of

1-10 diluted curds/100 g. dry mix) was added as an additional inoculum at the time of preparing the batter in certain experiments.

The composition (parts by weight) of the ingredients in all the methods was: black gram dhal or its flour, 100; parboiled rice semolina, 200; sodium chloride, 8·4 and water, 660.

Batter volume — As an index of the gas retaining capacity of the batter, its volume at any stage in the fermentation was measured.

Total gas production — The total volume of gas produced during fermentation referred to here as 'displaced volume' was measured by displacement of liquid paraffin[3].

Acidity — Twenty-five g. of the batter, suspended in 50 ml. of water, were titrated with 0·1*N* sodium hydroxide using phenolphthalein as indicator.

Non-protein nitrogen (*N.P.N.*) — Ten g. of batter were well mixed with 10 ml. of 10 per cent trichloracetic acid and nitrogen determined by the microkjeldahl method in an aliquot of the filtrate.

Reducing sugars — In the tungstic acid filtrate of the batter, by the Somogyi method[4].

Specific volume (*vol./wt*) *of the steamed Idli as softness index* — By displacement of kerosene, after coating the *Idli* with a thin film of wax to block the superficial air pockets with due correction in weight and volume for the coating.

Fermentation temperature — Fermentation was carried out at 23°, 31°, 36° and 41°C. For routine studies the batters were incubated at 29-30°C.

Criteria for judging fermentation — Acid production and volume increase were used since these could be measured easily and rapidly.

Viable microbial organisms — Five g. each of black gram flour and rice semolina were separately soaked in 15 ml. water and the total as well as yeast and fungal counts at 0 and 8 hr fermentation were determined employing plating technique. The microbial loads at 8, 13 and 20 hr of fermentation were measured by suspending the respective batters in 0·5 per cent sterile saline and plating out at appropriate dilutions. For total count, a medium containing 5·0 per cent peptone, 0·1 per cent dextrose, 0·5 per cent yeast extract and 1·5 per cent agar was used. Colonies were counted after incubation at 37°C. for 48 hr. Yeasts and fungi were plated out on dextrose-potato extract-agar medium[5]. The potato extract was prepared by boiling mashed potatoes in 5 parts of water for 1 hr and the extract filtered through cloth and immediately used for the preparation of the medium. The *p*H of the agar medium was adjusted to 3·5 just before dispersing it in the petri dish, by adding 3·5 ml. of 10 per cent sterile tartaric acid per 100 ml. of the medium. Counts of colonies of yeasts and fungi were made after incubation at 30°C. for 48 hr.

Share of bacteria and yeast in the fermentation — Penicillin G and chlortetracyclin hydrochloride were used for the selective inhibition of bacteria. In all the different types of experimental mixes, penicillin G (sodium salt; 20 mg. per cent or 33,400 I.U.) or chlortetracyclin hydrochloride (15 mg. per cent) was added to the batter and the acidity and gas retention in the batters compared against control sets.

Results and discussion

The values reported are averages of three different experiments. These replications were necessary to allow for differences from batch to batch due to natural variations. While reproducibility was generally good in any one set of experiments, variations ranging up to 20 per cent were observed in some extreme cases.

Criteria for following progress of fermentation — With the progress of fermentation in the wet grinding method, non-protein nitrogen and acidity in the batter increased while reducing sugars decreased up to 20 hr and then tended to increase (Table 1). Values measured at more frequent intervals (Table 2) showed that while the increase in acidity was noticeable even at the sixth hour, the increase in batter volume (i.e. gas formation) was observed only after about 10 hr, i.e. evidently when the strength of the fermentation acids was built up to a certain level.

The specific volume, an indication of the fluffiness of the *Idli*, increased as the fermentation progressed. But the method as used was found to be tedious and later experiments have also shown that small differences in quality could not be detected by this method. Detailed data relating to *Idli* quality as determined by this method have not, therefore, been reported here. Because of the ease of measuring acidity and batter volume, they were used as index of the progress of fermentation.

TABLE 1 — CHANGES IN FERMENTING *IDLI* BATTER (WET GRINDING METHOD)

(*Incubation temperature, 28-29°C.; values reported are for 25 g. of batter*)

Incubation period, hr	0	12	16	20	24	36	40
Non-protein nitrogen, mg.	3·5	6·5	8·1	8·8	8·9	10·8	11·8
Reducing sugars (as glucose), mg.	26	18	17	6	14	15	19
Acidity, ml. 0·1*N* NaOH	3·1	14·7	17·5	19·5	20·4	21·6	23·4

TABLE 2 — CRITERIA FOR FOLLOWING *IDLI* FERMENTATION (WET GRINDING METHOD)

(*Incubation temperature*, 28-29°C.)

Incubation period, hr	0	2	4	6	8	10	12	16	18	20
Acidity (per 25 g. batter), ml. 0·1*N* NaOH	3·2	3·4	3·7	6·4	8·0	10·6	12·8	16·8	17·6	19·0
Batter volume, ml.	200	200	200	200	200	280	300	350	400	425
Displaced vol.*, ml.	0	0	0	28	76	184	294	—	—	—
Specific vol.	1·19	1·30	1·37	1·41	1·43	1·40	1·40	—	—	—

*This value represents the volume of liquid paraffin displaced by the gas liberated from the batter at atmospheric pressure.

TABLE 3 — EFFECT OF TEMPERATURE ON FERMENTATION

Incubation period *hr*	Increase in acidity (*ml. of* 0·1*N NaOH*) per 25 g. batter at				Batter volume (*ml.*) at			
	23°C.	31°C.	36°C.	41°C.	23°C.	31°C.	36°C.	41°C.
4	0·4	0·7	1·3	1·6	200	200	200	200
6	0·7	0·9	3·3	5·6	200	200	200	225
8	0·9	2·9	8·2	12·3	200	200	275	360
12	0·9	8·8	18·6	22·0	200	340	400	425
16	3·1	14·7	26·7	—	200	400	400	—
18	3·4	17·5	27·0	—	225	400	400	—
20	4·7	15·9	26·2	—	250	420	425	—

Effect of temperature — Rate of fermentation increased with temperature in the range 23-41°C. (Table 3). At 23°C., the fermentation was slow; even after 20 hr the batter was under-ripe. At 30°C., however, the rate of fermentation was faster and about 16 hr were sufficient to give a ripe batter. The ripening time was about 12 hr at 36°C. and 10 hr at 41°C. Organoleptically, the *Idlis* made from batter fermented at 31°C. were soft retaining the full and characteristic aroma of *Idlis*, while those fermented either at 36° or 41°C., though soft, lacked the aroma. Presumably, the type of organisms developing at the higher temperatures and the course of fermentation are different and correspondingly the nature of the fermentation products contributing to the aroma also varies. Fermentation at temperatures around 30°C. is, therefore, to be recommended although this requires a longer period of fermentation. At temperatures below 25°C., the batter has to be fermented beyond 24 hr to get a ripe batter and good *Idlis*.

Comparison of dry mix with traditional wet grinding method — The dry mix flour when fermented for 12-14 hr, as is normally given to the batter in the traditional method, was found to give hard *Idlis*. In order to determine whether the presoaking of the black gram dhal for a period which precedes the grinding in the traditional method is the factor endowing good quality on *Idli* obtained by this method, acidity and batter volume at different periods of the fermentation, as also organoleptic quality as evaluated by a tasting panel of *Idlis* prepared by steam-cooking for 10 min. were determined on batters obtained by the following four different methods: (i) the traditional wet grinding process, (ii) the dry mix without any added inoculum, (iii) ' flour presoaking method ' where finely ground black gram flour was made into a paste with water, incubated for 6 hr at 30°C. and then mixed with rice semolina and (iv) the dry mix containing 20 mg. per cent dry yeast and mixed lactic inoculum in the form of curds. The composition of the major ingredients used for making these batters was the same and has been mentioned earlier. Data presented in Table 4 show that fermentation in the wet ground method was adequate to give soft *Idlis*, whereas when fermented for the same period, i.e. 12 hr (reckoned the from time of soaking the dry mix), acid and gas production in the dry mix method was very low and the *Idlis* were hard. It is, however, significant to note that when fermentation was continued for another 6-8 hr the batter from the dry mix method attains the level of fermentation as in the wet ground method and gives equally soft *Idlis*. Since this period of 6-8 hr corresponds roughly to the time of soaking given to the black gram dhal prior to grinding, it appeared that certain fermentative changes are initiated during the soaking of the black gram dhal and that the presoaking period may have to be considered as part of the fermentation itself.

This is confirmed by data presented in Table 5, relating to ' flour presoaking ' method. Here black gram flour made into a thick paste with water was allowed to undergo autofermentation for 6-8 hr and then the rice semolina was added. The fermentation

TABLE 4 — COMPARISON OF TRADITIONAL WET GRINDING AND DRY MIX METHODS

(*Incubation temperature*, 28-29°C.)

	Duration of fermentation, *hr*							
	0*	3	6	9	12	17	19	21
Increase in acidity (per 25 g. batter), ml. 0·1*N* NaOH								
Wet ground	0·0	1·1	1·6	2·9	8·8	—	—	—
Dry mix	0·0	0·0	0·0	0·0	3·1	5·5	6·4	8·2
Batter vol., ml.								
Wet ground	200	200	200	275	325	—	—	—
Dry mix	200	200	200	200	250	290	325	350

*The zero hour represents the time of mixing rice semolina to the ground black gram dhal in wet grinding method and the time of preparing the batter in dry mix.

TABLE 5 — COMPARATIVE STUDY OF DIFFERENT METHODS OF FERMENTATION

(*Incubation temperature*, 30-31°C.)

Treatment	Increase in acidity (*ml.* 0·1*N NaOH*) per 25 g. batter at				Batter vol.* (*ml.*) at				*Idli* quality at			
	15 hr	18 hr	20 hr	24 hr	15 hr	18 hr	20 hr	24 hr	15 hr	18 hr	20 hr	24 hr
Dry mix	10·4	11·4	12·0	14·9	550	650	700	700	NA†	NA†	NA†	NA†
Dry mix + yeast + curds	17·4	18·5	22·8	28·0	375	400	425	450	A, S	A, S	NA‡	NA‡
Wet ground	11·0	14·3	15·4	16·3	350	375	400	400	NA, H	A, S	A, S	A, S
Flour presoaked	12·3	16·4	18·7	17·4	475	520	550	550	NA, S	A, S	A, S	A, S

*Initial vol. 200 ml. †Very sour. ‡Bad odour. NA = not acceptable; A = acceptable; S = soft; H = hard.

proceeded at about the same rate as in the 'wet grinding' method. Thus, if this fermentation time of the black gram component before adding the rice is also allowed for, the batter from the dry mix behaves similar to batter obtained by wet grinding. There is also an indication that the presence of rice along with black gram flour from the start of the fermentation somewhat alters the type of fermentation. This can be corrected by the addition of yeast and curds as inoculum to the dry mix.

The organoleptic quality of the *Idlis* prepared from the dry mix was poor. Gas formation in this method proceeded somewhat faster than in the other three methods and often in an uncontrolled manner leading to development of foul smell in the *Idlis*. The addition of curds as an inoculum completely eliminated this smell; the lactic organisms presumably prevented the growth of unwanted types of microflora. Since the 'flour presoaking' method, where no external inoculum is necessary, and the dry mix, where both yeast and curds are added as inoculum, give soft and acceptable *Idlis* and do not involve the wet grinding of the constituents, they are capable of being developed as practical methods for *Idli* making to replace the traditional wet grinding method. Details are under publication elsewhere.

Microbial growth during fermentation — When black gram flour and rice semolina were soaked separately for 8 hr, there was an increase in the total count of organisms (Table 6) but yeast and fungal count registered an increase only in the case of black gram flour and not with rice semolina. The rice semolina, when soaked separately, fermented to a very small extent. Its contribution to acidity and gas production in *Idli* fermentation, therefore, appears quite small as compared with that of the black gram component. These results are further confirmed by later observations that antibiotic-resistant organisms played a major part when black gram flour alone is fermented.

TABLE 6 — VIABLE MICROORGANISMS PRESENT IN FERMENTING INGREDIENTS

(*Incubation temperature*, 29-30°C.)

	Total count per g. of batter at		Yeast and fungal count per g. of batter at	
	0 hr	8 hr	0 hr	8 hr
Black gram flour	24×10^3	160×10^3	$2{\cdot}0\times10^2$	40×10^2
Rice semolina	$2{\cdot}4\times10^3$	350×10^3	$2{\cdot}6\times10^2$	$2{\cdot}2\times10^2$

When mixtures of rice and black gram dhal or flour were fermented, the total microbial count increased with the progress of fermentation in all the four different treatments (Table 7). In the treatment where yeast had been added to the dry mix, there was

TABLE 7 — MICROBIAL LOAD IN FERMENTING *IDLI* BATTERS

(*Incubation temperature*, 29-30°C.)

Treatment	Total count per g. of batter at			Yeast and fungal count per g. of batter at		
	8 hr	13 hr	20 hr	8 hr	13 hr	20 hr
Dry mix	4.4×10^6	100×10^6	1340×10^6	8.5×10^3	12.2×10^3	9.2×10^3
Dry mix + yeast + curds	3.2×10^6	15×10^6	180×10^6	9.0×10^3	9.6×10^3	30×10^3
Wet ground	0.45×10^6	7.8×10^6	144×10^6	15.8×10^3	20×10^3	6.4×10^3
Flour presoaked	3×10^6	8×10^6	130×10^6	15×10^3	14×10^3	5.6×10^3

TABLE 8 — INFLUENCE OF ANTIBIOTICS ON ACID AND GAS PRODUCTION

(*Incubation temperature*, 30-31°C. *for* 24 *hr*; *initial batter volume in all cases*, 150 *ml.*)

Treatment	Increase in batter vol. *ml.*	Increase in acidity per 25 g. batter *ml.* 0·1*N NaOH*	Inhibition in acid production %
Dry mix			
Control	285	20·9	—
Mix + penicillin G*	nil	3·8	82
Mix + chlortetracyclin*	nil	3·1	85
Dry mix + yeast + curds			
Control	265	18·3	—
Mix + penicillin G	20	7·5	59
Mix + chlortetracyclin	15	6·8	63
Wet ground mix			
Control	125	18·8	—
Mix + penicillin G	nil	3·6	81
Mix + chlortetracyclin	nil	3·8	80
Flour presoaking process			
Control	270	20·0	—
Mix + penicillin G	30	6·0	70
Mix + chlortetracyclin	25	6·2	69
Black gram flour†			
Control	300	15·6	—
Flour + penicillin G	30	11·5	26
Flour + chlortetracyclin	25	11·0	29
Rice semolina†			
Control	nil	3·7	—
Semolina + penicillin G	nil	0·1	97
Semolina + chlortetracyclin	nil	0·1	97

*Penicillin G sodium salt was added at 20 mg. per cent level (33,400 I.U.) or chlortetracyclin hydrochloride was added at 15 mg. per cent level to the batter.

†Fifty g. of black gram flour or rice semolina were incubated with 165 ml. water.

a continuous increase in the yeast and fungal count up to 20 hr of fermentation while in the other three cases, a decrease in the viable yeast count was registered between 13 and 20 hr of fermentation.

Effect of antibiotics — Addition of antibiotics (Table 8) to the fermenting batter was found to inhibit the fermentation significantly, the extent of inhibition varying with the different methods of fermentation. The inhibition of acid production, for instance, was about 83, 80, 70 and 60 per cent respectively in the dry mix, wet ground, flour presoaking and yeast added dry mix batters. When black gram flour alone was fermented, the inhibition was only about 25 per cent indicating that, in this case, major portion of the fermentation was due to antibiotic-resistant organisms belonging to the yeast and fungi group. This was true to a smaller extent in the yeast added dry mix and flour presoaking methods. Gas production also followed generally a similar pattern of inhibition though the extent of inhibition was much higher than in the case of acid production.

It may at first appear from these data that in normal *Idli* fermentation, the action of yeasts is a minor one. In a mixed fermentation where both bacteria and yeasts take part and yeast action is symbiotic needing substances released by the bacteria, the antibiotics may indirectly affect yeast growth.

Role of black gram and rice in the fermentation — When black gram flour and rice semolina were separately soaked in water and autofermented, the acid production and the increase in batter volume were high in the case of black gram flour, but quite small in the case of rice (Table 8) indicating that the major component contributing to the fermentation is the black gram flour. In the presence of antibiotics the fermentation was slowed down in both cases although in the case of black gram flour the action of organisms belonging to the yeast and fungi group was predominant. Further work in apportioning the relative contribution of black gram and rice components to the fermentation is in progress.

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Quantitative Estimation of Carbohydrates by Paper Partition Chromatography

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A simple micromethod for the quantitative estimation of aniline hydrogen phthalate (AHP) stained sugar spots has been standardized. Of the solvents examined for their effective elutability, besides formamide, the following are found suitable within 50-80 per cent concentration range : acetic acid, methanol, ethanol, and acetone. The various factors that affect the accuracy and reproducibility of results in this method have been critically examined. As a result of the analysis of some sugar mixtures, using three different solvent systems, it is found that the overall error does not exceed ±6 per cent.

IN the course of our work on the characterization of the polysaccharides elaborated by *Vibrio cholerae*[1], it became necessary to evolve a simple and accurate chromatographic method for the separation and estimation of carbohydrate mixtures. Ever since Patridge[2] used paper partition chromatography for the separation and detection of sugars, many workers have reported their quantitative estimation by (1) extraction and determination by a suitable micromethod[3-5], (2) staining and reading the coloured spots by direct photometry[6-8] and (3) elution followed by photocolorimetric estimation[9-11].

Barr[12] claimed that the elution of the coloured spots formed by heating aniline hydrogen phthalate (AHP) with glucose on filter paper and its colorimetric estimation would give more accurate results than that obtained with direct photometry and would also consume less time than elution followed by microestimation. As pointed out earlier[13], the use of glacial acetic acid as an eluent in the above method is not the ideal thing (cf. Blass[14]). In view of this, the usefulness of some of the common organic solvents as eluents has now been studied with reference to AHP reagent under different conditions, and a simple and accurate method has been described for the estimation of sugars in a mixture.

Experimental procedure

D-Glucose, D-galactose, D-glucuronic acid, D-arabinose and L-rhamnose (10-100 μg. in 0·02 ml. of solution) were applied at 1·5 in. intervals on a line drawn 3·5 in. away from one end of a 20 × 22 in. Whatman No. 1 sheet and irrigated by the usual descending technique in a Chromatocab with the following solvents: *n*-butanol-acetic acid-water (4: 1: 1), phenol saturated with water and *n*-butanol-pyridine-benzene-water (5: 3: 1: 3).

The chromatograms were generally run overnight at room temperature (28-34°C.), air dried, sprayed with the AHP reagent[15] on both the sides and heated at 105°C. for 5-60 min. Squares of coloured spots (1·5 × 1·5 in.) were cut out along with the control strips and eluted by intermittent shaking in test tubes for different time intervals with 5 ml. of the following organic solvents under varying conditions: acetic acid, acetone, benzene, chloroform, dioxan, ethanol, ethyl acetate, ethyl methyl ketone, formamide, methanol and tetrahydrofuran.

The optical density of the eluates was determined in a Klett-Summerson photoelectric colorimeter using blue filter (420 mμ).

All the reagents and sugars employed were of analytical grade.

Results and discussion

The suitability of eleven organic solvents for extracting AHP stained glucose (50 μg.) spots from filter paper strips was examined using *n*-butanol-acetic acid-water solvent. In Table 1 are recorded the intensities of colour eluted at 1, 2 and 3 hr intervals in terms of colorimeter readings. The extracted colour values increased with time in some cases, within the 3 hr limit examined, especially in acetic acid solvent. From the signs shown in the remarks column, based upon the 3 hr observation, it would be seen that acetic acid, ethanol and methanol

TABLE 1 — COMPARATIVE EFFICIENCIES OF SOLVENTS

(50 μg. *glucose-AHP stained spots eluted at different intervals*)

Eluent	1 hr	2 hr	3 hr	Remarks*
Acetic acid	84	116	120	+++
Acetone	21	27	26	+
Benzene	0	0	0	0
Chloroform	2	7	9	0
Dioxan	18	17	20	+
Ethanol	74	78	78	+++
Ethyl acetate	11	11	12	+
Ethyl methyl-ketone	25	26	21	+
Formamide	60	61	60	++++
Methanol	75	79	79	++++
Tetrahydrofuran	36	46	46	+++

*The degree of elution is indicated by the following signs in an increasing order: 0 (no elution), +, ++, +++ and ++++.

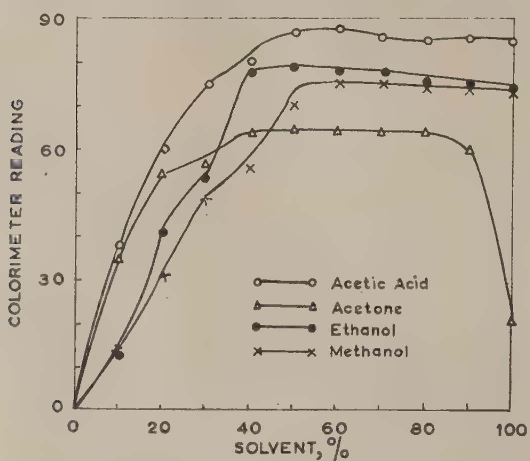

FIG. 1 — INTENSITY OF COLOUR EXTRACTED BY DIFFERENT CONCENTRATIONS OF SOLVENTS, EXPRESSED IN TERMS OF COLORIMETER READINGS, AFTER 1 HR INTERVAL (50 μG. GLUCOSE)

along with formamide should be promising. The suitability of formamide has already been reported[13]. In order to achieve complete elution the above three solvents in different combinations with water, viz. 10-90 per cent, were studied, using 50 μg. of glucose and 1 hr contact. The results are graphically presented in Fig. 1. Even though absolute acetone did not give encouraging results (Table 1), some concentrations of aqueous acetone were tried and found useful (Fig. 1). A similar observation, with reference to ninhydrin stained amino acid spots, was made by Giri[16] who found that the best elution was obtained with 75 per cent ethanol but not with absolute alcohol.

A close examination of Fig. 1 suggests: (1) The extent of extraction of colour by varying concentrations of eluents is different; (2) the maximum colour intensity is obtained with acetic acid; and (3) in all these solvents the maximum intensity is attained in about 50 per cent composition and remains constant up to 100 per cent concentration, except in the case of acetone, wherein there is a sudden decline after 90 per cent.

It would appear from the graphs (Fig. 1) that 50-100 per cent concentration of these solvents, with the exception of pure acetone, should be able to completely elute the colour from the filter paper strips. Actually, the entire extraction was possible only within the range of 50-80 per cent. It was,

TABLE 2 — INTENSITIES OF COLOUR EXTRACTED BY DIFFERENT ELUENTS

Eluent*	Glucose conc., μg.					
	10	20	40	60	80	100
Acetone	15	30	64	96	128	156
Ethanol	14	32	67	100	135	164
Methanol	15	32	65	95	117	160

*70% aqueous solution.

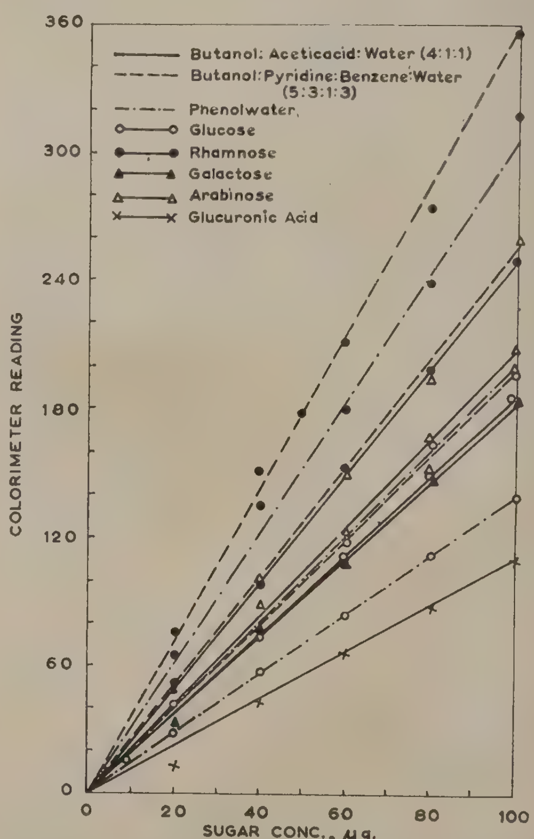

FIG. 2 — STANDARD GRAPHS FOR SUGARS OBTAINED WITH 50 PER CENT ACETIC ACID AS ELUENT IN DIFFERENT SOLVENT SYSTEMS

however, observed that pure methanol would elute the colour completely.

With a view to examining the quantitative relationship between the amount of sugar and the intensity of the colour extracted, 70 per cent (arbitrarily chosen) aqueous ethanol, methanol and acetone were studied as eluents. The results (Table 2) suggest that colour intensity obeys Beer-Lambart's law within the range 10-100 μg. of carbohydrate concentration. The linearity of the above relationship was also noticed when arabinose, glucose, galactose, glucuronic acid and rhamnose were irrigated with *n*-butanol-acetic acid-water, phenol-water and *n*-butanol-pyridine-benzene-water solvents and eluted with 50 per cent acetic acid for 1 hr (Fig. 2). Generally the colour intensities of the eluates for the same amount of carbohydrates varied from one another. For instance, when chromatograms were run in *n*-butanol-acetic acid-water solvent, the colour intensities of 60 μg. of arabinose, glucose, glucuronic acid and rhamnose were 123, 112, 67 and 150 respectively in 50 per cent acetic acid (Fig. 2). The colour intensities also depended upon the nature of the irrigating solvent. Maximum values were obtained in *n*-butanol-pyridine-benzene-water system followed by phenol-water and *n*-butanol-acetic acid-water solvents for most of the sugars. Among the three solvents it was found that good compact bands were obtained with the solvent containing pyridine.

With a view to finding out the optimum conditions for the elution of coloured sugar spots developed with AHP reagent, the following factors were studied using glucose or galactose.

Duration of heating — The AHP sprayed chromatograms were heated for different intervals, viz. 5, 10, 15, 20, 30, 40, 50 and 60 min. The intensities of the colour eluted remained constant between 10 and 30 min.; it reached its maximum after 40 min., beyond which a fall was noticed.

Duration of irrigation — Filter paper sheets were irrigated for varying periods, the stained bands eluted and their optical densities compared. It was found, for example, in the case of 100 μg. galactose that the Klett readings were 220, 230 and 264 respectively for 6 hr, 10 hr and overnight irrigations. It may be concluded that the maximum intensity of colour under these conditions was obtained in the case of chromatograms run overnight, wherein the solvent had overflown. Further, it was observed that the intensities of the colour eluted from spots containing equal amounts of the same sugar depended upon the area of the spot. This remark finds support in the theoretical observations of Brimley[17]. Giri[16] reported similar results with reference to the estimation of amino acids stained with ninhydrin.

Time taken for the elution of the coloured spots — With a view to ascertaining the minimum time necessary to completely elute 100 μg. galactose coloured spot with 50 per cent acetic acid, colorimeter readings were taken at different intervals. It was noticed that the elution was over after 10 min. and remained constant for 24 hr. Similar results were also obtained with 70 per cent methanol, ethanol and acetone.

In the light of the above findings the following conditions would be helpful in obtaining reliable and reproducible results:

(1) The chromatograms of the standard as well as the sample sugars should be developed under similar conditions every time.

(2) The AHP sprayed chromatogram should be heated at 105°C. for 15 min. and then eluted with any one of the following solvents within the concentration range of 50-80 per cent: acetic acid, acetone, ethanol and methanol.

(3) Even though the minimum time of elution is 10 min., for safety, the strip might be kept in contact with the solvent for 30 min.

TABLE 3 — ANALYSIS OF CARBOHYDRATE MIXTURES

Mixture No.	Carbohydrate components, μg.				
	Glucuronic acid	Glucose	Galactose	Arabinose	Rhamnose
1	—	40 (0, 0, −5·0)*	—	60 (+1·6, +5·8, +1·6)	—
2	—	50 (−2·0, +6·0, −4·0)	—	50 (+4·0, +6·0, 0)	—
3	37·5 (−4·0, —, —)	—	25 (0, —, −4·0)	—	37·5 (+1·0, —, +1·0)
4	—	20 (0, −5·0, −5·0)	—	40 (+5·0, 0, −5·0)	40 (0, 0, −1·3)
5	—	—	40 (+5·0, —, —)	30 (+6·6, —, —)	30 (0, —, —)
6	44 (−2·3, —, —)	—	44 (+2·3, —, —)	56 (+5·3, —, —)	56 (5·4, —, —)

*The order of the percentage error given within parentheses refers to the following solvents: referred to in the same order, *n*-butanol-acetic acid-water (4:1:1), water-saturated phenol, and *n*-butanol-pyridine-benzene-water (5:3:1:3).

(4) It was found necessary to cork the test tubes during the period of elution with a view to preventing the evaporation of the solvent.

In order to test the accuracy of this method, a few mixtures of sugars were prepared and analysed for the amounts of carbohydrates present in them. Three different solvent systems were employed for irrigation and 50 per cent acetic acid was used as an eluent. The results, along with the percentage errors, are shown in Table 3. Generally the error does not exceed ±6 per cent (Table 3).

The utility and applicability of these solvents in the quantitative elution of the coloured spots developed with established spraying reagents[9,18,19], other than AHP, are under investigation.

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Meiotic Abnormalities in *Gagea reticulata* Schultes

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The cytological behaviour of *Gagea reticulata* Schultes collected during the normal (February) season and late in the season (May) has been investigated. The meiotic behaviour of chromosomes in plants collected during February has been found to be quite normal whereas plants collected during May have been found to show a number of meiotic irregularities such as non-pairing of chromosomes, early disjunction, fragmentation, presence of bridges and laggards with variable number and sizes, resulting in polyspory. The meiotic irregularities recorded are probably due to exposure to high temperature during May.

PREVIOUS cytological investigations on the genus *Gagea* were confined to 8 species[1]. Recently, Malik and Sehgal[2] contributed to the cytology of one more species, *Gagea reticulata* Schultes. The present communication embodies observations on meiotic abnormalities in this plant.

Materials and methods

A few plants of *G. reticulata* were collected from Hoshiarpur (Punjab) growing late in the season (May). The flower buds were fixed in a solution of one part of acetic acid and three parts of absolute alcohol. The fixations were also done in Carnoy's fixative (6: 3: 1). The anthers from fixed material were squashed in iron acetocarmine. Pollen fertility was judged from the percentage of pollen grains which stained in acetocarmine.

Results

G. reticulata collected during the month of February from the above locality exhibited a normal course of meiosis. Pairing of the chromosomes was

perfectly normal and 12 bivalents were counted at diakinesis and metaphase I. This was followed by a regular A-I, where 12 univalents were seen at either pole. The second meiotic division essentially showed no departure from the usual course and resulted in a high degree of fertility.

In plants exhibiting abnormal meiosis 10 per cent P.M.C.'s showed a normal course as detailed above. The normal and abnormal P.M.C.'s occurred side by side. In some mother cells, 9 bivalents and 6 univalents were seen (Plate I, Fig. 1). Figs. 2-4 depict mother cells with variable number of bivalents and univalents. The occurrence of varying number of univalents in different P.M.C.' scan be attributed to the failure of pairing during prophase I. Fig. 5 presents interesting features. It shows 4 bivalents and 16 univalents. In addition, there are 3 nucleoli present. Fig. 6 depicts a pollen mother cell with 11 bivalents and 2 univalents at metaphase I. Apparently, the occurrence of 2 univalents may be due to non-pairing or an early disjunction of one of the bivalents. Fig. 7 exhibits a mother cell where some of the bivalents have undergone fragmentation. In some of the cells, at metaphase I, few chromosomes and fragments remained outside the equatorial plate (Fig. 8). Often, chromosomes formed stretching threads and assumed peculiar configurations. Sometimes P.M.C.'s were encountered where bivalents formed a compact mass and it was difficult to analyse individual bivalents. Similar situations were recorded by Johnsson in *Alopecurus myosuroides*[3] and recently by Malik and Tandon in *Asparagus currilus* Ham.[4]. Such an abnormality has been attributed to disturbed nucleic acid metabolism[5].

At anaphase 1, the distribution of these teeming chromosomes to the poles was random and unequal. There were usually $\frac{12}{12}$, $\frac{12}{11}$, $\frac{8}{8}$, $\frac{12}{9}$, $\frac{7}{7}$, distributions. The remaining univalents lagged and were lying astray in between the two poles. However, bivalents were never seen to lag. Rarely P.M.C.'s were noticed where 24 univalents constituted one group or lay scattered throughout the mother cell. Sometimes lagging univalents misdivided resulting in the formation of half univalents (Figs. 9 and 10). As a result of such laggardism, there was marked disparity both in quantity and quality of nuclei at T-I (Fig. 11). Furthermore, the lagging chromosomes failed to reach the poles (Fig. 12) and later became incorporated as micronuclei of variable size and number. Bridges were also noticed (Fig. 13). They were usually unaccompanied by acentric fragments. The number of such bridges varied from 1 to 4 and even persisted at telophase I. The formation of such bridges may be caused by delayed disjunction or persistence of interstitial chiasmata. Such bridges may break at any point and as such are likely to cause deficiencies and duplication. After the completion of telophase I, the two daughter nuclei divided. Sometimes the division in one or both nuclei failed. This resulted in the formation of triads and diads (Figs. 14 and 15). At telophase II, many half univalents as well as chromatic bits were noticed in between the two poles. As a result of all these abnormalities quantitatively equal or unequal tetrads, pentads and even configurations above this level were found (Figs. 16 and 17). Monads (Fig. 18) were also recorded which were evidently formed due to the failure of both the divisions. As a result of the irregularities described above, the pollen grains formed were extremely variable in size. Even stainable grains varied in diameter.

Discussion

The meiotic behaviour of chromosomes of plants collected during the normal season (February) was found to be quite normal. A number of meiotic abnormalities were, however, recorded in plants collected late in the season (May). These irregularities could not be due to fixation, since the same fixative was used in both the cases.

Several workers have artificially induced many meiotic abnormalities like desynapsis, stickiness, laggardism, non-reduction and formation of diploid spores. Sax has observed several such abnormalities by the application of hot and cold treatment in *Tradescantia paludosa*[6]. Gustafsson and Nygren have also recorded the temperature effect on pollen formation and meiosis in *Hieracium robustum*[7]. Similar chromosomal aberrations are also reported to be caused by various environmental conditions. Matsuura and Haga[8] reported that normal meiosis occurred in *Trillium kamstschaticum* during winter under snow cover. When these plants were grown in warmer temperature, they showed a breakdown in meiosis. The course of meiosis was normal in buds of *Gagea reticulata* plants collected during the month of February. The abnormalities described above were from plants collected during the month of May and were apparently due to the reaction of the plant to the environmental factors. Since these irregularities were observed in the hot months of May, it is reasonable to assume that extreme heat may be responsible for the disturbances in meiosis. In this connection, it is pertinent to compare the average maximum temperature in these months at Hoshiarpur, which during February was 18°C. and during May 38°C. It is tempting to suggest that the course of meiosis in flowers of plants collected in May was modified due to high temperature to which the plant is ordinarily not adapted and ever since the observations of Malik and Tandon[4,9] in *Asparagus curillus* Ham. and

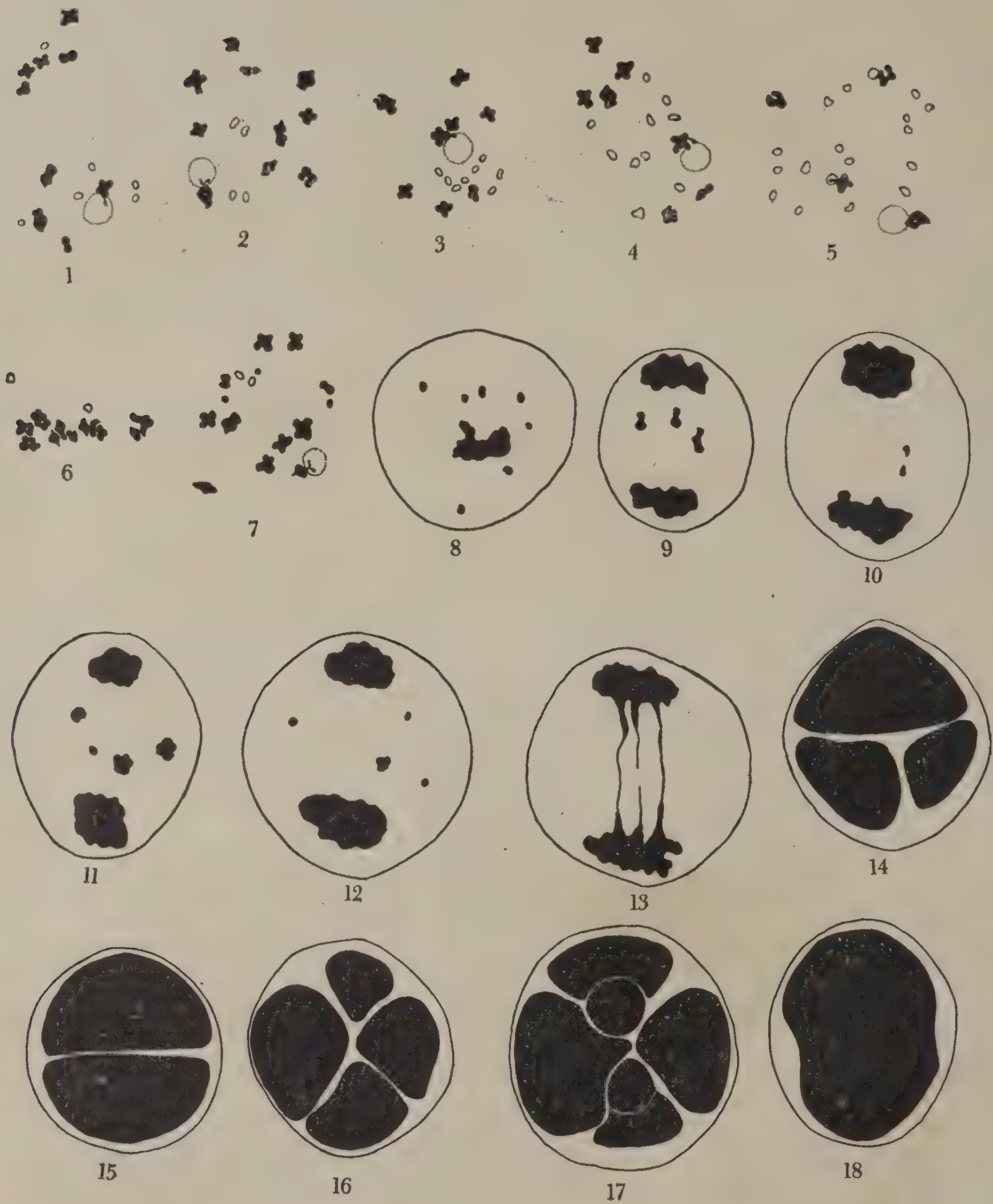

PLATE I — MEIOTIC ABNORMALITIES IN *G. reticulata* SCHULTES × 720. [Figs. 1-5: P.M.C.'s with variable number of bivalents and univalents. Fig. 6: Metaphase I with 11 (II) + 2 (I). Fig. 7: 11 (II) + 2 (I) and 3 small fragments. Fig. 8: Few chromosomes remaining outside the equatorial plate. Figs. 9-12: Telophase I with laggards. Fig. 13: P.M.C.'s with 3 chromatic bridges. Fig. 14: Triad. Fig. 15: Dyad. Fig. 16: Tetrad with unequal sized microspores. Fig. 17: Hexad. Fig. 18: Monad]

Suaeda fruticosa Forsk., it has become increasingly clear that there is a correlation between temperature and meiotic irregularities. Raman and Krishnaswami[10] also observed meiotic abnormalities in *Sorghum halepense* collected during hot months. Their investigations revealed that these meiotic disturbances were apparently caused by summer heat. Khoshoo has reported a similar situation in *Sisymbrium irio*[11].

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Letters to the Editor

HEAT TOLERANCE COEFFICIENT IN *MURRAH* BUFFALO CALVES

Mean heat tolerance coefficient of 12 (6 male and 6 female) *murrah* buffalo calves between 6 and 12 months of age has been determined and found to be 85·15 ± 6·25. Significant differences have been observed in the heat tolerance coefficient between animals (within sex) and highly significant differences between months (age groups). No significant difference has been observed in the heat tolerance coefficient of male and female calves. High air temperature and relative humidity lower the ability of the calf to withstand thermal stress. It has been found that 1°F. rise in atmospheric temperature causes as much change in heat tolerance coefficient as that due to 1 per cent change in relative humidity.

Very few studies have been made on the heat tolerance of young cattle[1-4]. The authors, finding no reference in literature with regard to heat tolerance in buffalo calves, thought it worthwhile to undertake this study to find out the differences, if any, in heat tolerance between animals and between animals of different sex and between months (age groups), and to determine the effects of environmental factors, if any.

The heat tolerance coefficient of six male and six female *murrah* buffalo calves, between 6 and 12 months of age, was calculated for each month (age group) according to Rhoad[5], to express the complex physiological value in a single factor. The management of these calves during the experimental period has been described in detail by Taneja and Bhatnagar[6]. Maximum and minimum air temperature and relative humidity were recorded each day.

The mean heat tolerance coefficient of these calves during the year under study was found to be 85·15 ± 6·52 (S.D.). These variations have been statistically analysed[7].

The results of analyses given in Table 1 indicate significant differences in the heat tolerance coefficients between animals of different sex and highly significant differences between months (animals of different age groups). This suggests that the significant differences of heat tolerance coefficients between animals may be due to differences in their genetic make up for their adaptability and highly significant differences between months (animals of

TABLE 1 — ANALYSIS OF VARIANCE

Sources of variation	D.F.	M.S.	Variance ratio
Between animals (within sex)	10	15·97	2·10*
Between months (age groups)	11	450·43	5·94†
Between sex	1	28·43	2·69
Between months × sex	11	10·55	1·39
Between animals (within sex) × months	110	7·59	—

*Significant at 5% level.
†Significant at 1% level.

different age groups) may be purely due to environmental (mostly climatic) factors.

Air temperature and relative humidity are considered responsible, as a result of this study, for the variation in the heat tolerance coefficient between months (animals of different age groups). The partial correlation coefficients, between air temperatures and heat tolerance coefficient, and between relative humidity and heat tolerance coefficient, are found to be negative and highly significant, being —0·323 and —0·292 respectively. These values indicate that high relative humidity and high air temperature lower the ability of the calf to withstand thermal stress. Multiple correlation coefficient has been found to be highly significant being 0·425 and the multiple regression equation for heat tolerance coefficient is

$$Y = 88{\cdot}56 - 0{\cdot}0258X_1 - 0{\cdot}0321X_2$$

where Y is the estimated heat tolerance coefficient, X_1, air temperature and X_2, relative humidity.

The equation reveals that 1°F. rise in air temperature causes as much change in heat tolerance coefficient as 1 per cent change in relative humidity.

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PREPARATION OF UBIQUINONE (COENZYME Q) FROM RICE BRAN

Ubiquinone has been isolated from the lipid-soluble concentrates of the rice bran, in an yield of 40 mg./100 g. of the rice bran concentrates. The identity of ubiquinone has been confirmed through the study of its ultraviolet absorption spectra as well as the spectra of the potassium borohydride reduced product.

Ubiquinone belongs to a family of lipid-soluble quinones widely distributed in animal tissues[1,2]. Crystalline preparations of the quinone obtained from beef heart and pig heart have been assigned a coenzyme-like function in the terminal electron transport of biological oxidation-reduction systems[3,4]. Although the vitamin status of these compounds is still uncertain, their ubiquitous occurrence in mitochondria of all tissues examined so far indicates their participation in some phase of oxidative metabolism.

A compound with spectral characteristics different from heart ubiquinone, but which could replace it in the biological test system, has been isolated from cauliflower buds, alfalfa and spinach leaves[5,6]. Other than these reports, there is very little information, however, on the content of these quinones in cereals and pulses which form the bulk of Indian dietaries. A recent communication by Page *et al.*[7] deals with the distribution of ubiquinone in the foodstuffs used for rearing laboratory animals. Out of the few vegetables and other foodstuffs examined by these authors, only corn oil was found to be a good source of the quinone though not as rich as animal tissues.

An integrated process for the preparation of a tocopherol-rich oil, a vitamin B complex concentrate and inositol has been developed in this laboratory based on rice bran as the raw material[8]. Separation of α-tocopherol from the other isomers of the vitamin was attempted by chromatography on activated magnesium phosphate-Hyflo Supercel columns and elution by ethyl ether in petroleum ether[9]. By increasing the ethyl ether concentration in the solvent, the mixture resolved itself into α-tocopherol (60 per cent), γ-tocopherol (30 per cent) and two other smaller fractions which have not been identified. The initial few fractions in this elution schedule, however, responded negatively to the Emmerie and Engel test for tocopherols[10], but showed absorption with $\lambda_{max.}$ in the region 265 to 285 mμ ($\lambda_{max.}$ for α-tocopherol in alcohol is 292 mμ). On removal of the solvent from these fractions, a yellow oil separated out which yielded brownish yellow crystals at —15°C. In spectral properties in the ultraviolet region, the residue was reminiscent of ubiquinone; attempts were, therefore, made to process the unsaponifiables of rice bran oil essentially according to the procedure developed by Crane *et al.*[11] for the isolation of ubiquinone.

Rice bran concentrate (100 g.) prepared by the CDRI process[8] was refluxed for 30 min. in 500 ml. 95 per cent ethanol containing 25 g. pyrogallol and 100 g. potassium hydroxide. After cooling, the saponified material was mixed with 250 ml. water and extracted thrice with 200 ml. portions of petroleum ether (b.p. 50-60°C.). The ether extracts were combined, washed free from alkali, dried over anhydrous sodium sulphate and distilled under vacuum to give a viscous residue (yield 4 g.). This was taken up in 20 ml. isooctane and the solution kept at —15°C.

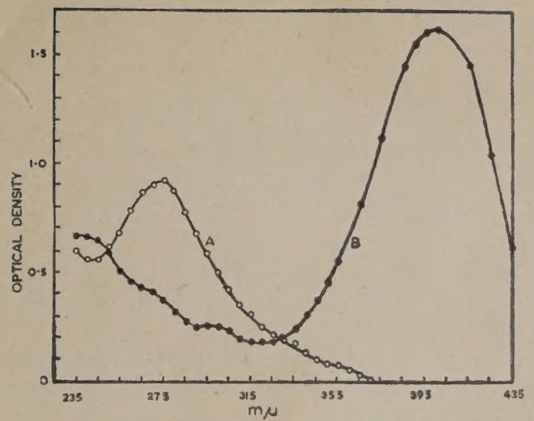

FIG. 1 — ABSORPTION SPECTRUM OF UBIQUINONE FROM RICE BRAN

After standing at this temperature for three days, a whitish precipitate separated out which was removed by centrifugation in the cold. The clear yellow supernatant was passed through a Decalso column (18·5 × 3·3 cm.) previously washed and equilibrated with isooctane. After charging the material, the column was washed with 250 ml. isooctane and the washes discarded. Elution of the quinone was achieved by passing 500 ml. of 5 per cent (vol./vol.) ethyl ether in isooctane. The eluates were collected in 100 ml. fractions. The first and the last fractions were rejected and the middle three combined. The solvent was removed by distillation *in vacuo* and residue dispersed in 10 ml. hot ethanol and the insolubles removed by quick filtration. The ethanolic solution was allowed to dry under vacuum (yield 40 mg.).

The absorption spectrum of this compound in absolute ethanol is reproduced in Fig 1. $\lambda_{max.}$ of the oxidized form was 275 mμ giving an extinction coefficient value ($E^{1\%}_{1\,cm.}$) of 200. On reduction with potassium borohydride an intense yellow colour appeared, the absorption at 275 mμ was considerably lowered, a slight hump appeared in the region of 288 to 296 mμ and a very sharp peak at 390 to 400 mμ. In cyclohexane $\lambda_{max.}$ of the oxidized form was 272 mμ. Reversed phase chromatography, according to Lester and Ramasarma[12], was employed to resolve the oxidized compound. Chromatography was carried out on silicone grease-impregnated paper (instead of Corning silicone fluid No. 550[12]) using isopropanol : water : : 3 : 1 as the mobile phase. After development for 18 hr, the paper was treated with potassium borohydride followed by 0·1*N* hydrochloric acid. An intense yellow spot with an R_f of 0·85 appeared indicating that the compound was apparently homogeneous. Further chemical and biological studies on this compound as well as similar quinones isolated from other cereals and pulses are in progress to elucidate their relation to ubiquinone and will be reported later.

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VERTICAL DISTRIBUTION OF TWO COMMON SHIPWORMS OF VISAKHAPATNAM HARBOUR

Studies on the vertical distribution of the two common shipworms, *Teredo furcillatus* and *Bankia campanellata*, of Visakhapatnam harbour have shown that attack by these two organisms is concentrated near the surface of the test panels and maximum attack is observed near the low water mark; attack by *B. campanellata* has been found to increase with increase in the depth of water.

VERTICAL ZONATION IS ONE OF THE STRIKING FEATURES of littoral faunas and the subject has attracted the attention of many workers. However, only few observations are available on the vertical distribution of wood-boring molluscs[1-6].

The present note deals with the vertical distribution of two common shipworms, *Teredo furcillatus* and *Bankia campanellata*, of Visakhapatnam harbour as no work has been done so far on these borers.

Ten panels, each 5 by 5 in. and 0·5 in. in thickness, of deal wood were suspended at the naval base, from high tide level up to 15 ft below the low water mark. The blocks were so arranged that there was a distance of 2 ft between two panels. The results are presented in Table 1.

TABLE 1 — VERTICAL DISTRIBUTION OF SHIPWORMS IN VISAKHAPATNAM HARBOUR

(Counts of T. furcillatus were made on panels immersed between 8 March and 9 April 1956, and that of B. campanellata between 21 November and 26 December 1956. Mean low water level was 5 ft)

Block No.	Depth from high tide level ft	*T. furcillatus*	*B. campanellata*
1	2*	51	40
2	{4*, 5*}	47	48
3	6	60	52
4	8	40	54
5	10	26	63
6	12	14	56
7	14	15	66
8	16	14	75
9	18	15	74
10	20	17	73

*Intertidal.

With regard to the vertical distribution of different species of *Teredo*, a review of the literature reveals that the vertical zonation varies with different species. In the case of *T. norvegica* there is an increase in the intensity of infection with increase in depth[1]. In *T. pedicellata* also infestation is most abundant near the bottom[4]. Edmondson[7] at Hawaii found that *T. diegensis* and *T. bartschi* were concentrated near the surface and occurred only in limited numbers at the lowest levels. At Visakhapatnam harbour, *T. furcillatus* showed the same type of zonation as exhibited by *T. diegensis* and *T. bartschi*. Maximum attack was noticed near about the low water mark.

The attack of *B. campanellata* increased with increase in depth of water (Table 1). Similar observations have been made with the other species of *Bankia* (*B. gouldi* at Beaufort[2], *B. setacea* at Friday harbour, Washington[3], and *B. gouldi* at Chesapeake Bay, Maryland[5]).

These observations may be helpful in connection with the protection of timber against attack of shipworms.

I express my thanks to Prof. P. N. Ganapati, under whose direction the work was carried out and for his valuable suggestions.

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SECTION B

SECTION C

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